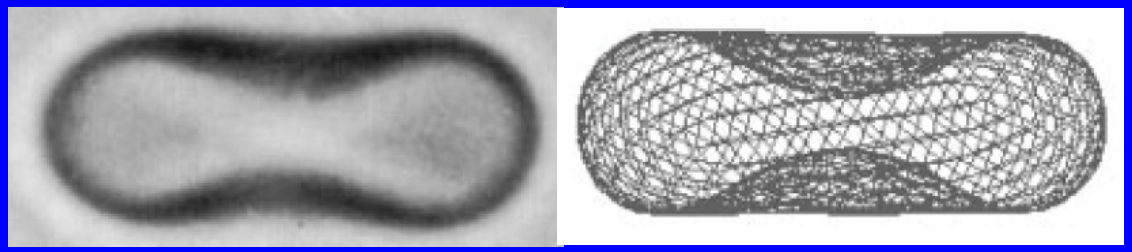
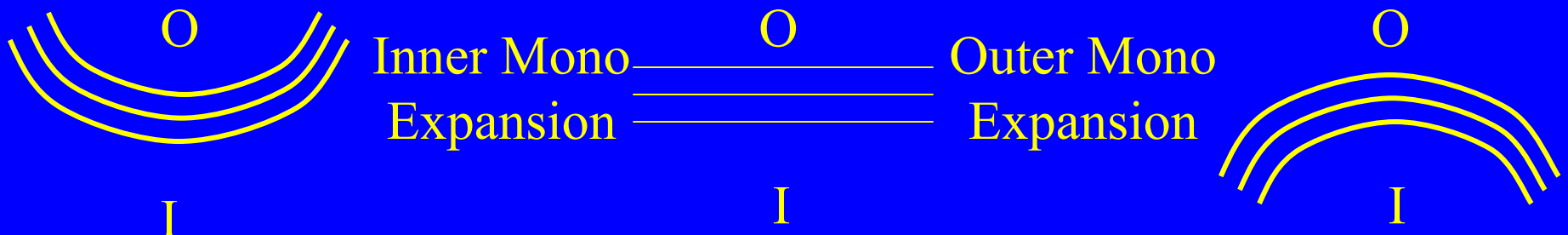
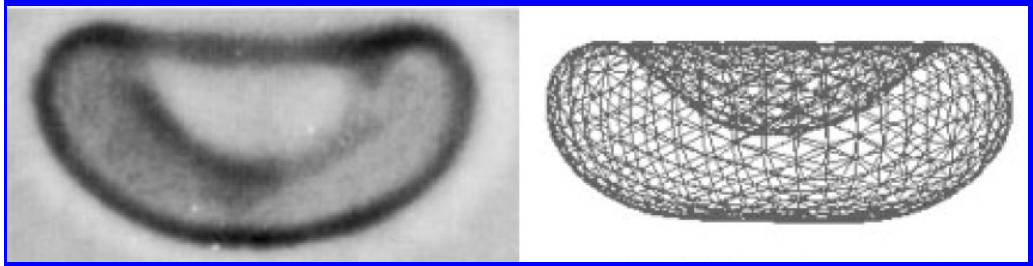
Biophysical Analysis of Membrane Functions by Laser Tweezers

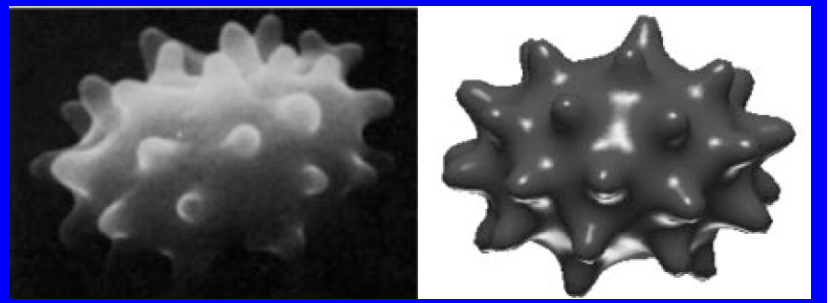
- Plasma Membrane Functions (Control by PIP2 Levels in Plasma Membrane)
  - Cell Motility
  - Endocytosis (Cell Volume regulation)
  - Membrane resealing
- Modified Model of Membrane Structure

Evidence for the Bilayer– Couple hypothesis from membrane mechanics



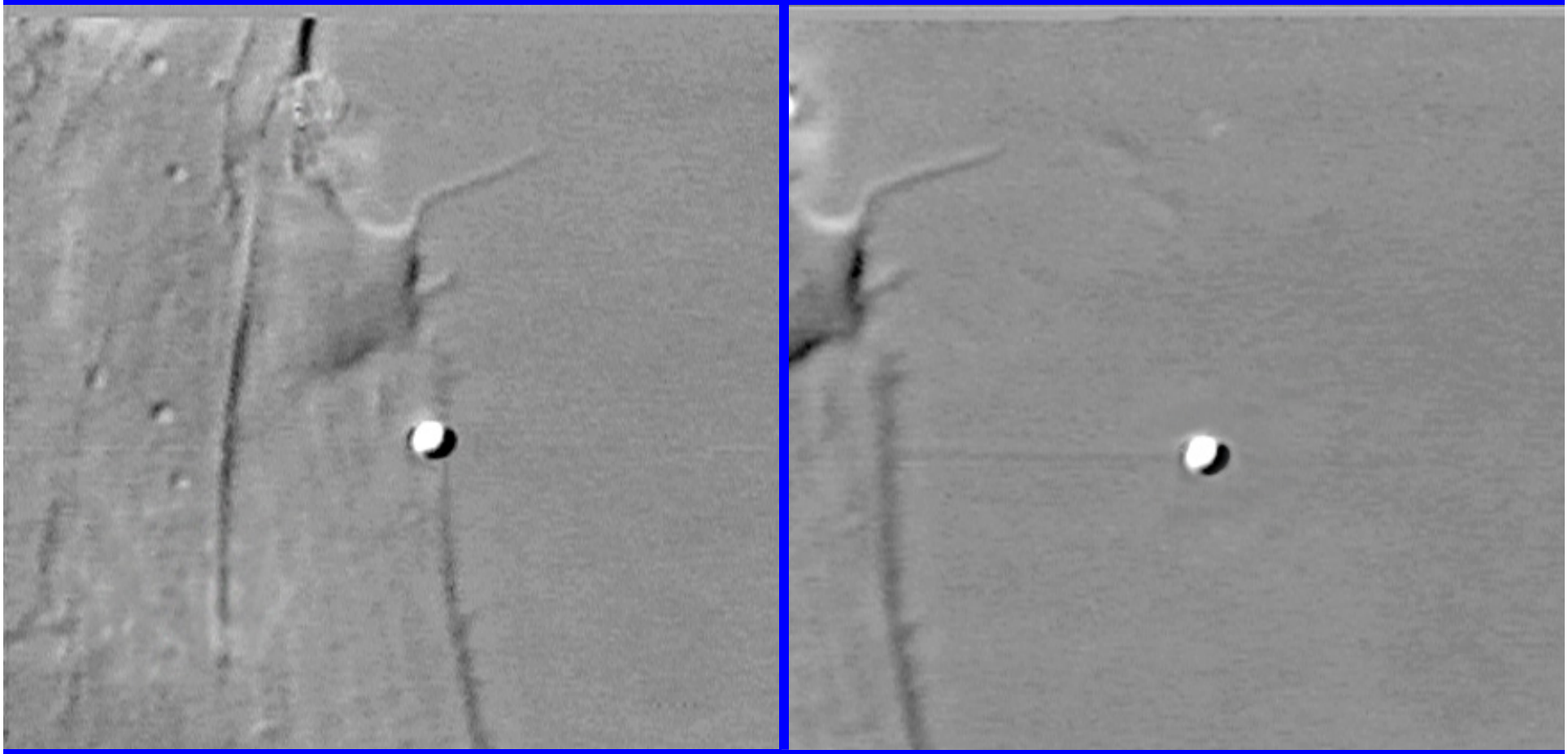




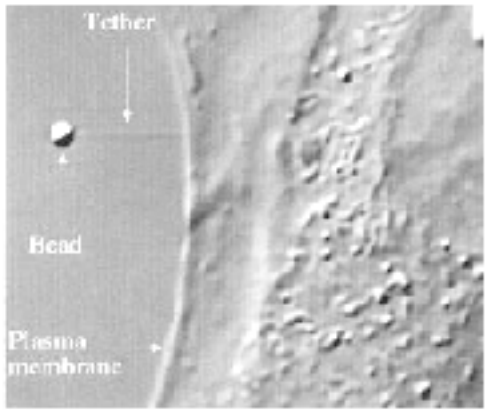


Lim et al., (2003) PNAS. 99:16766-9

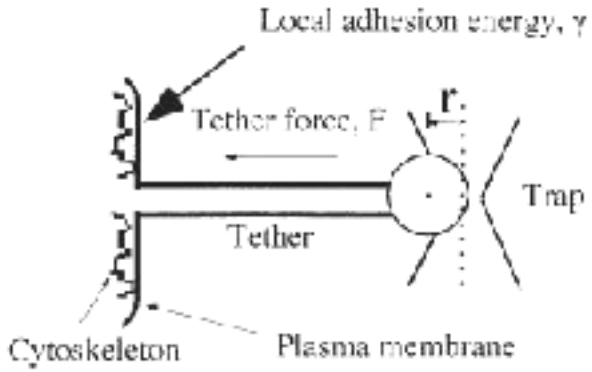
Tether Formation on Fibroblasts



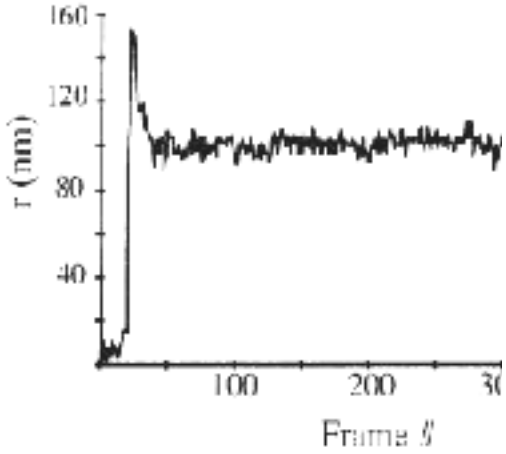
A


Tether
Bead
Plasma membrane

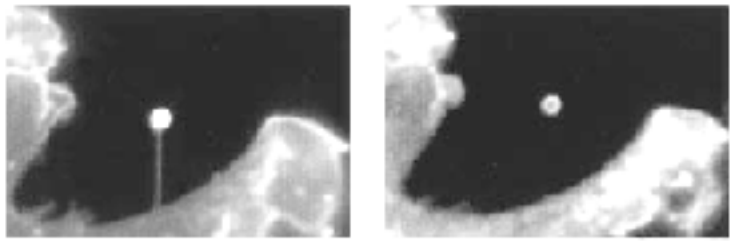
B


Local adhesion energy, γ
Tether force, F
r
Trap
Tether
Cytoskeleton
Plasma membrane

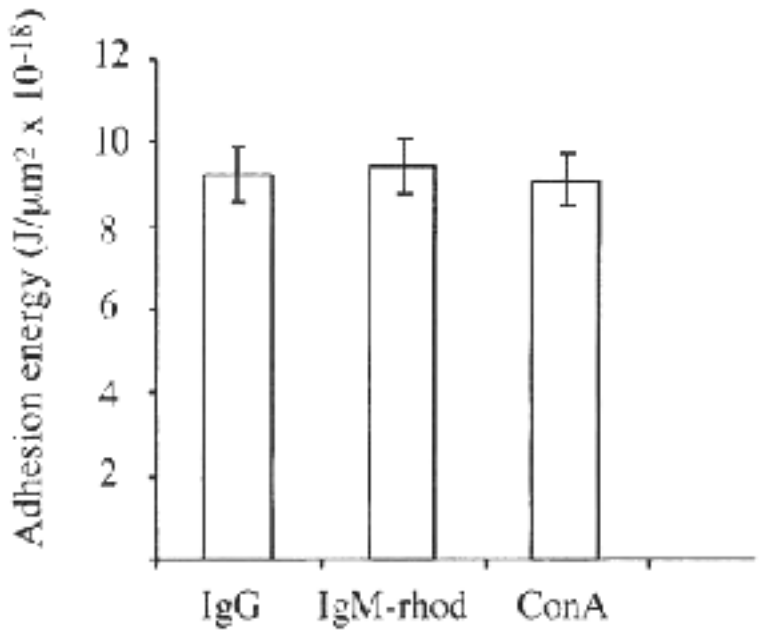
C


160
120
80
40
r (nm)
100
200
Frame #

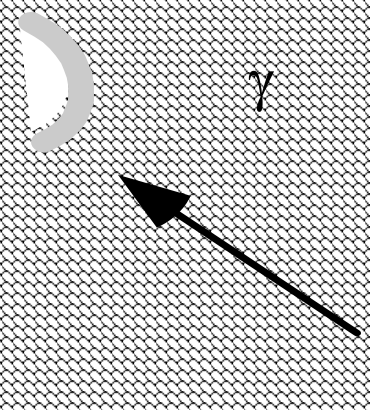
D



E

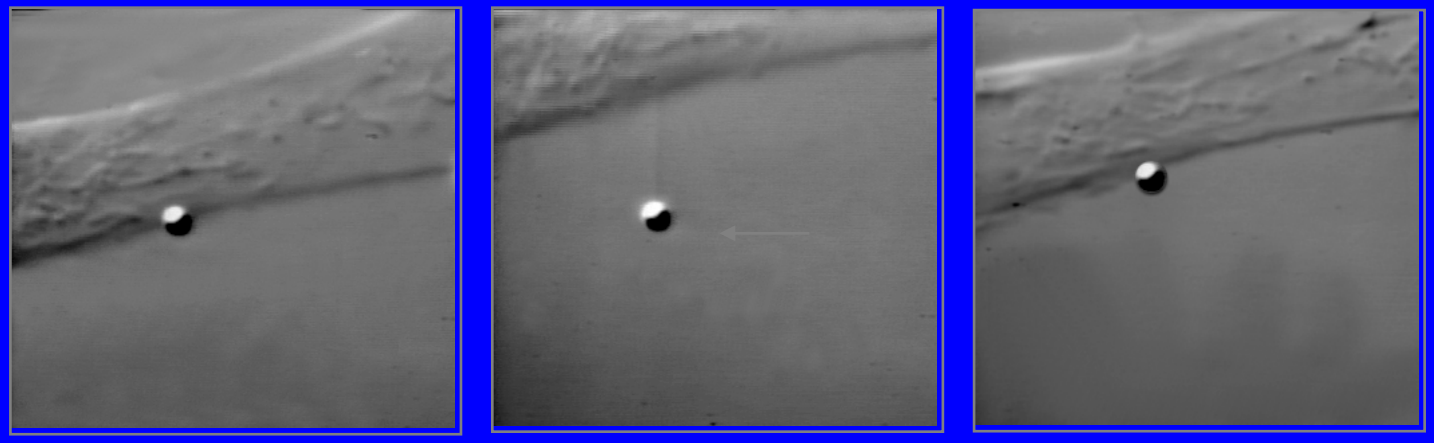

Adhesion energy (J/μm² x 10⁻¹⁸)
12
10
8
6
4
2
IgG
IgM-rhod
ConA

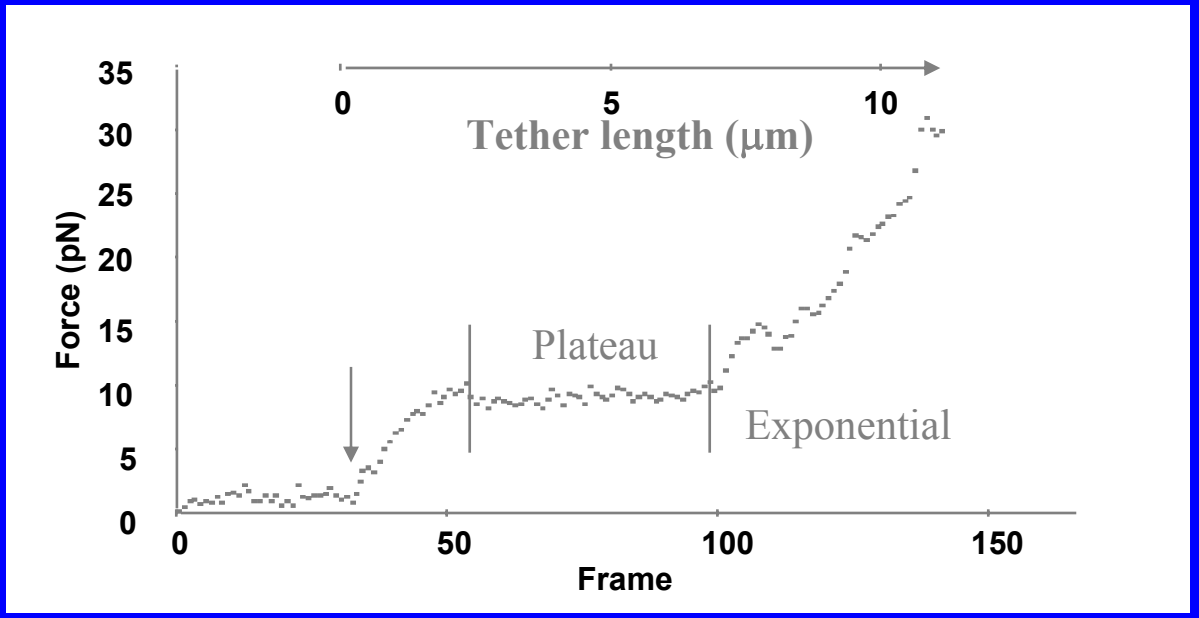
Apparent Membrane Tension



B: membrane bending stiffness

Tether Force vs. Length Indicates Membrane Reservoir is Present

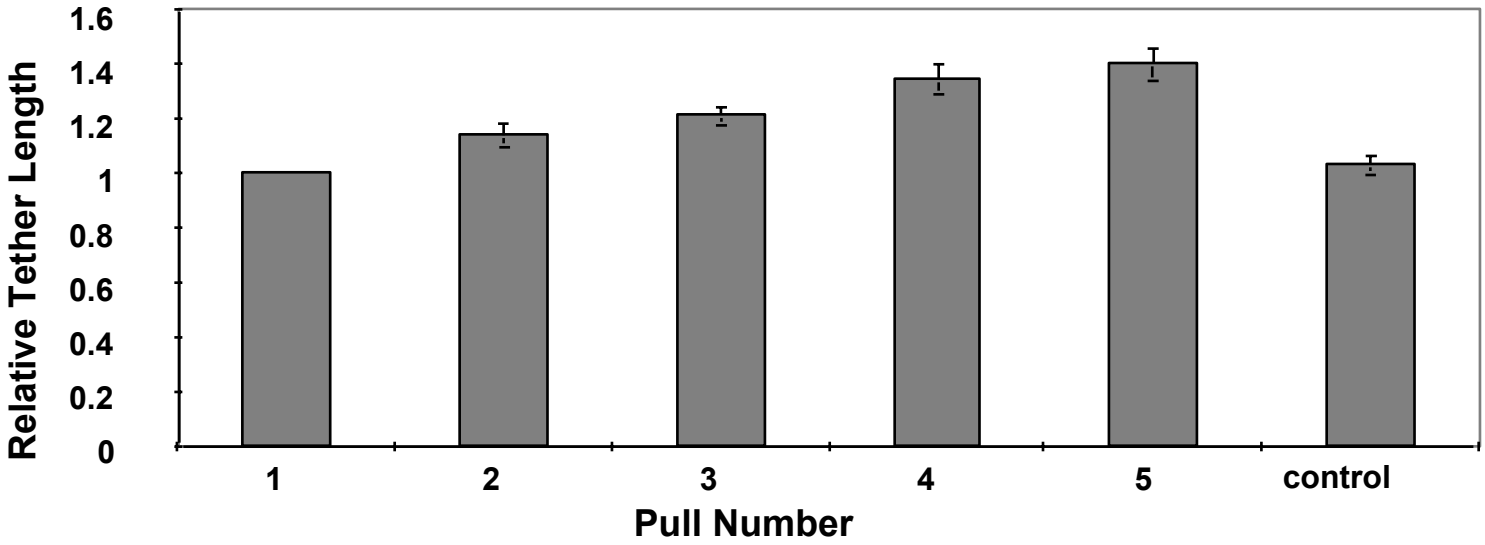




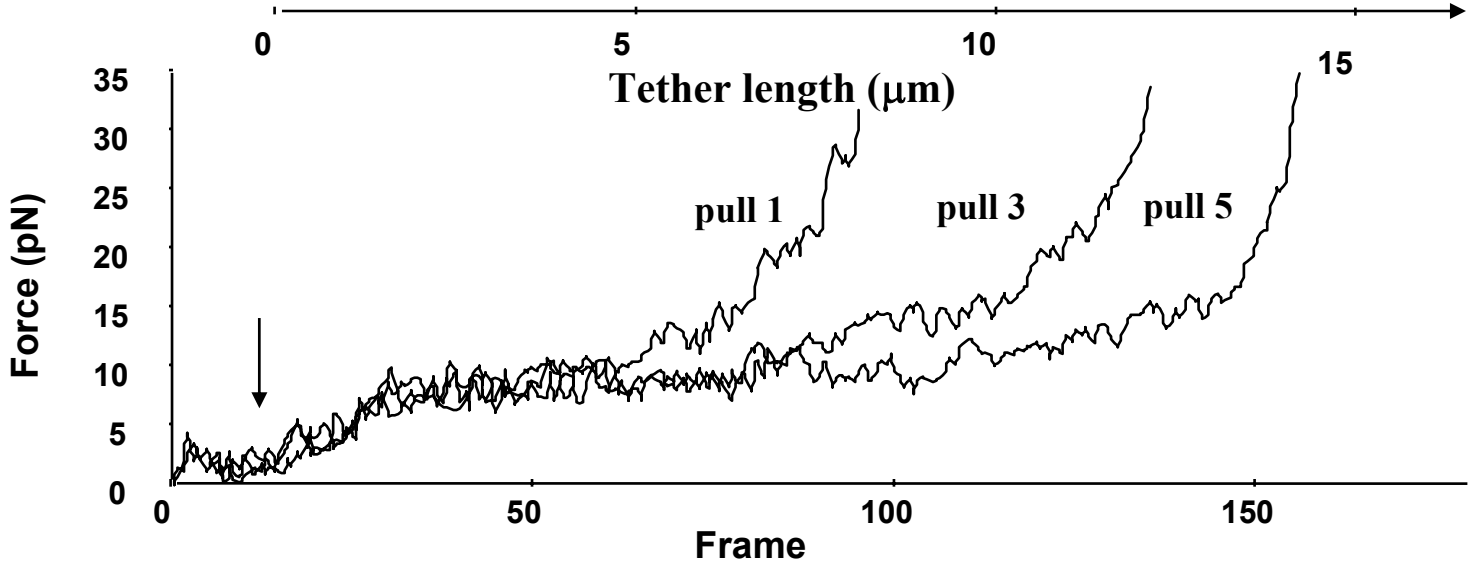
Raucher and Sheetz, Biophys J 77:1992 (1999).

Reservoir Increases With Each Tether Pull

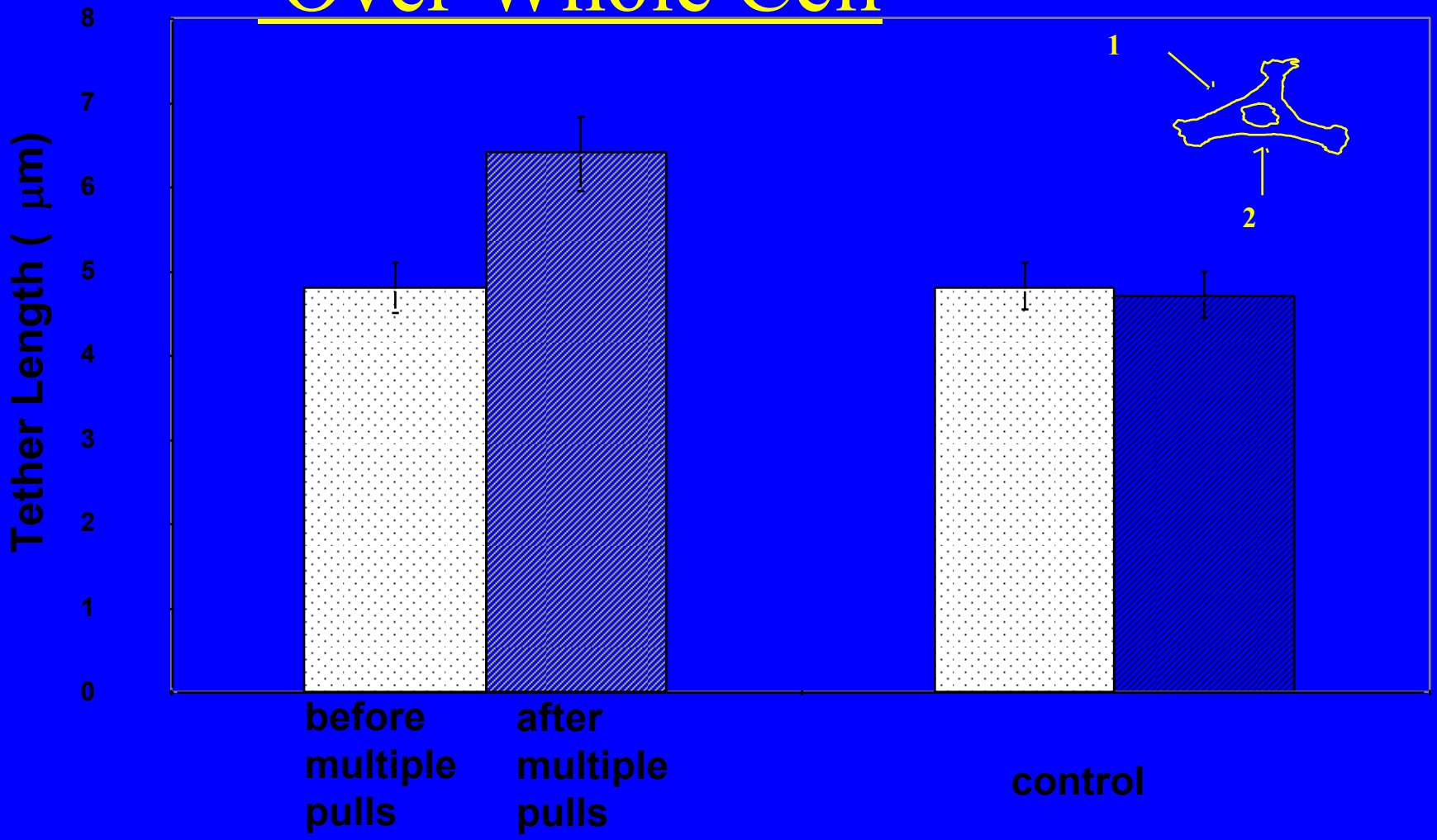
a)



b)



Reservoir Increase Is Sensed Over Whole Cell



Raucher & Sheetz (1999) Biophys. J. 77:1992.

Summary

- In-plane tension is small and is continuous over the whole cell surface

- Membrane-cytoskeleton interaction is the major component of the apparent membrane tension

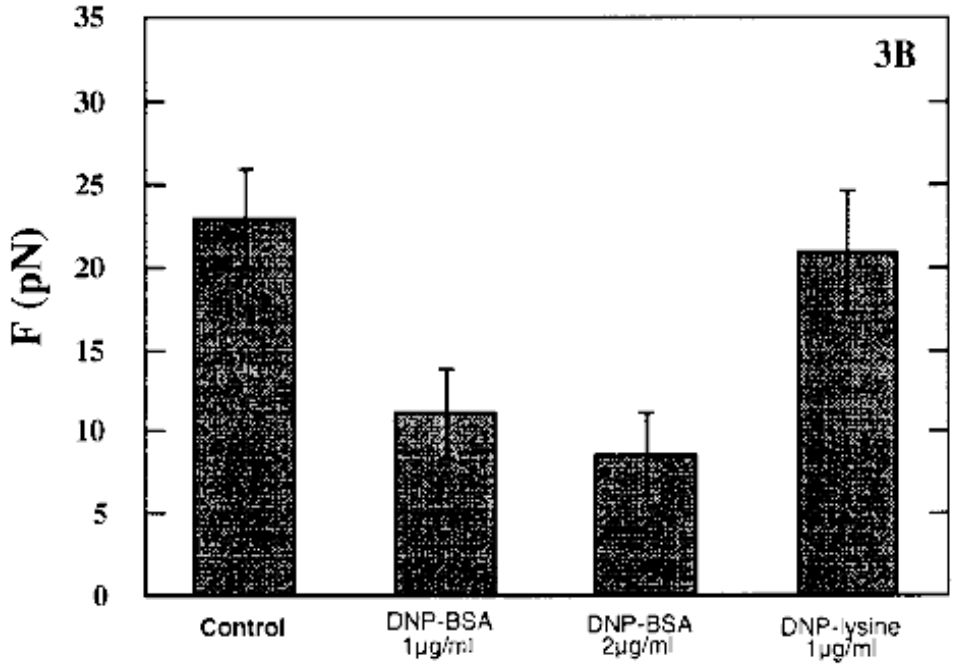
Cell Biophysics: Analysis of Physical Basis of Cell Functions

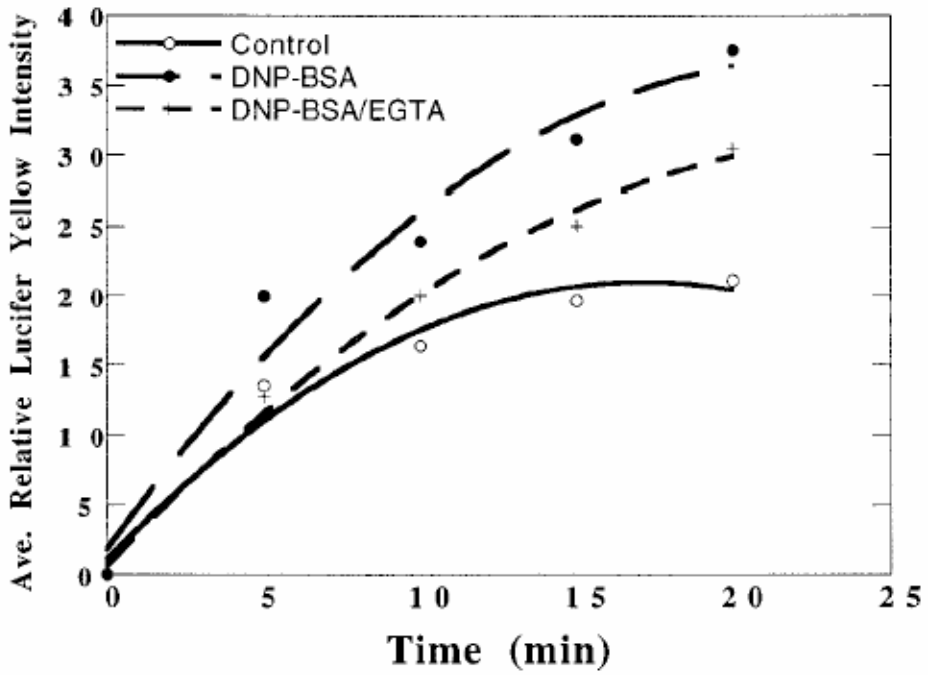
- Physical and chemical analysis of cell functions is critical
- Tension in Membrane is Critical for Membrane Functions (Global Control)
- Cell Forces Generated and Sensed by Cytoskeleton

Functions Controlled by Plasma Membrane Tension

- Motility
- Endocytosis Rate
- Cell Volume regulation
- Bleb resistance (Membrane-cytoskeleton adhesion)
- Membrane resealing

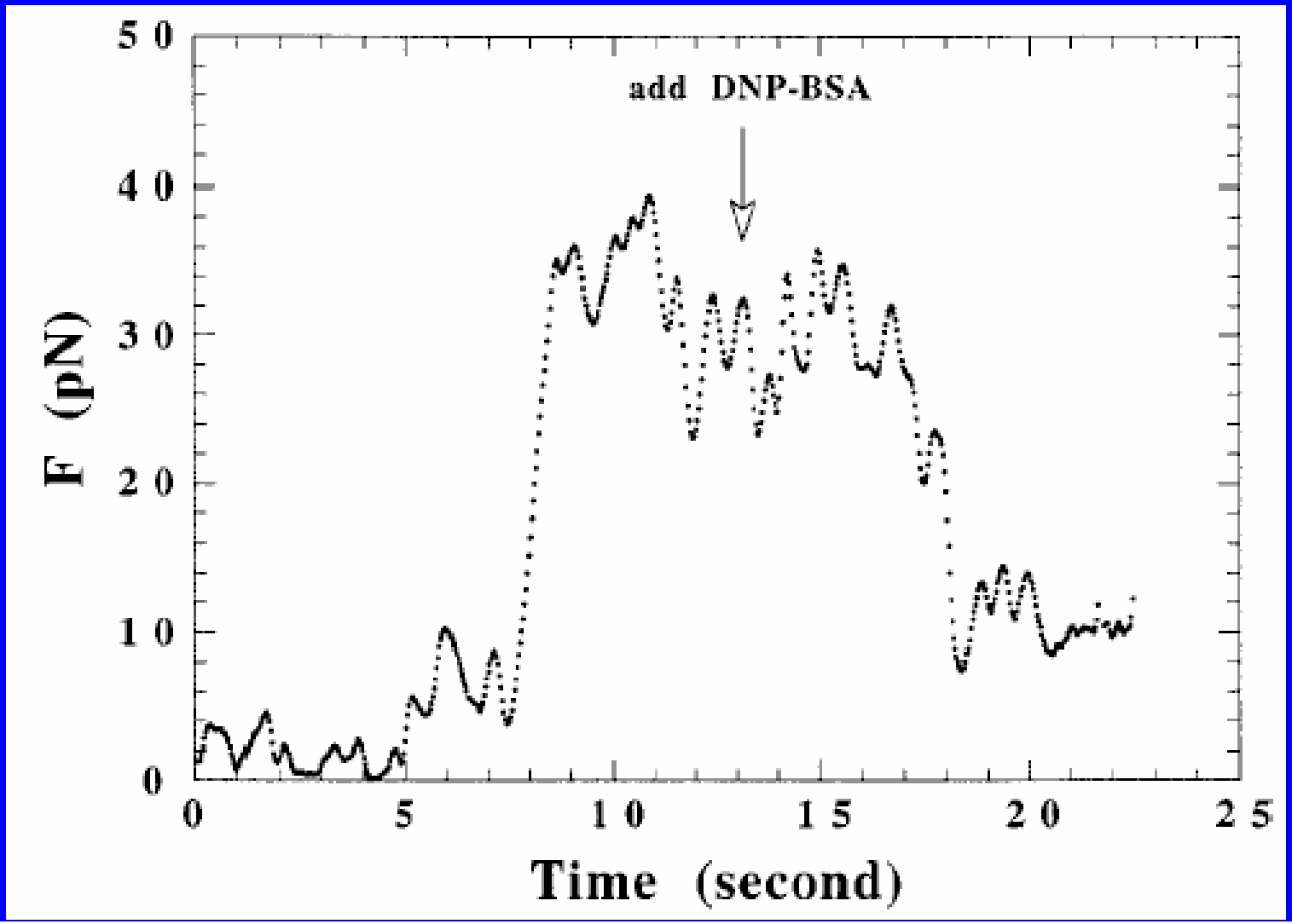
RBL Secretion Correlates with Drop in Tether Force and Rise in Endocytosis Rate



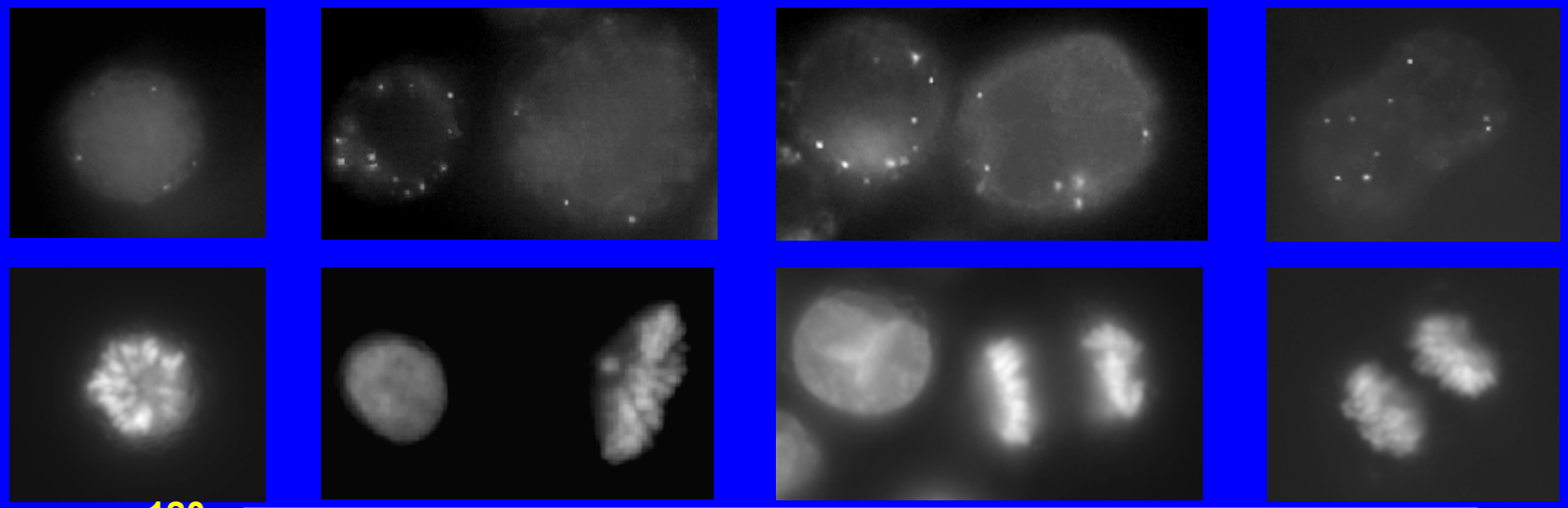


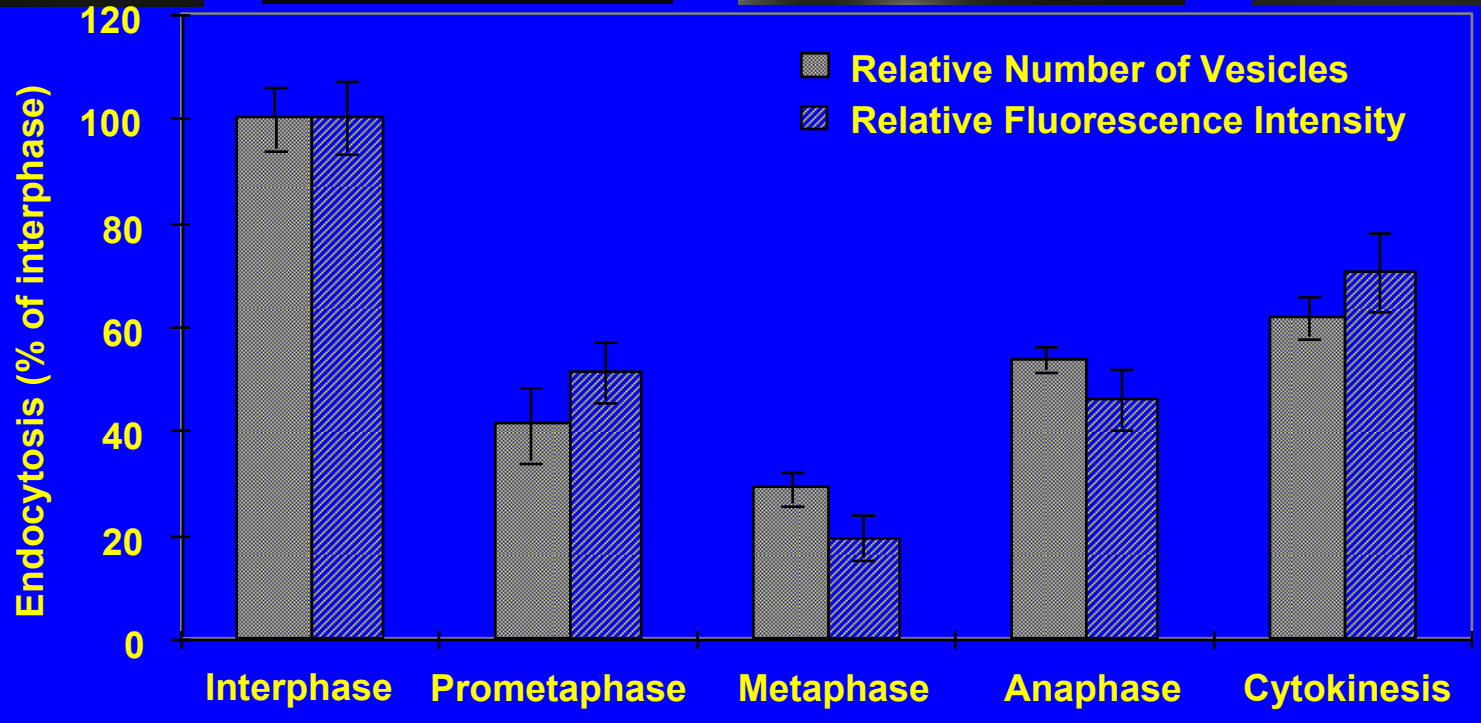
Dai et al. (1997) J. Gen. Physiol. 11:1

RBL Tether Force Decreases Faster Than Granule Secretion

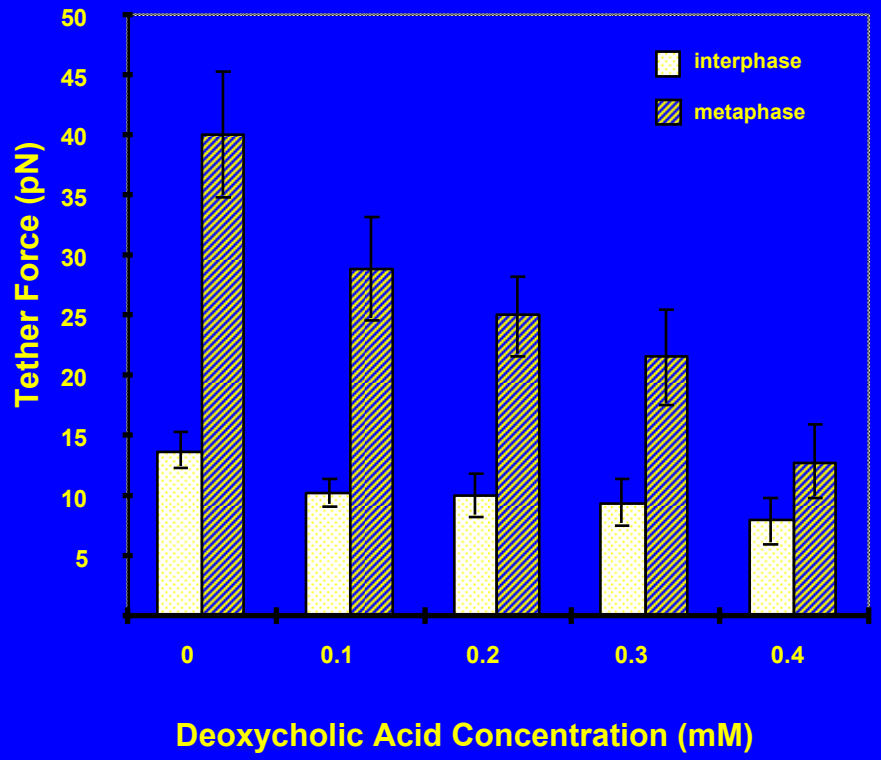


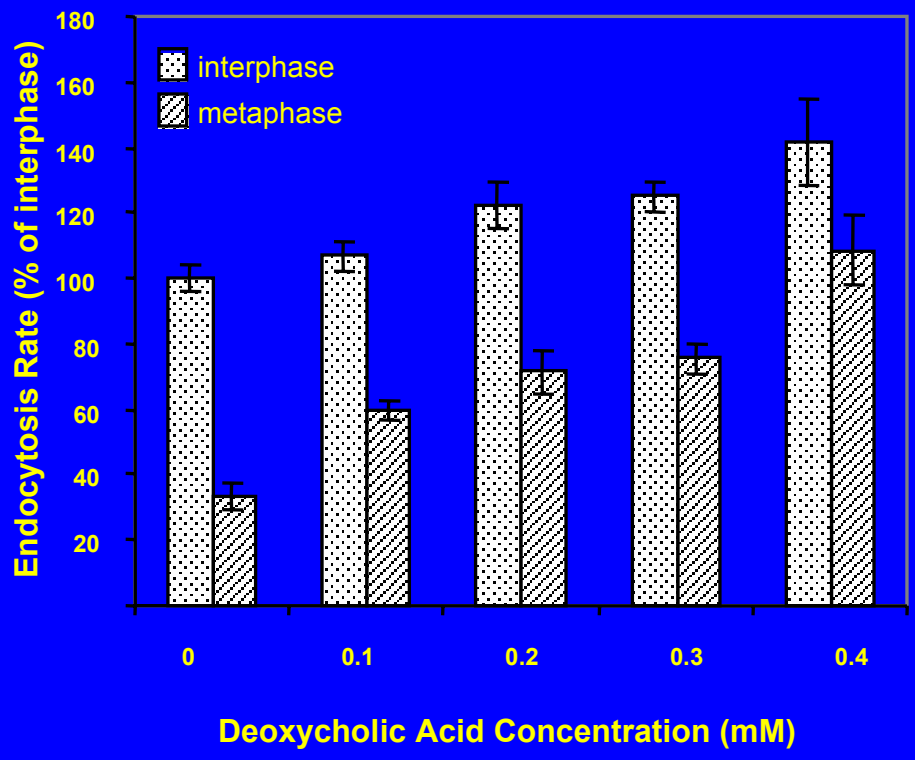
Endocytosis Inhibited Dramatically in Mitosis





Titration of Membrane Tension with Deoxycholic Acid

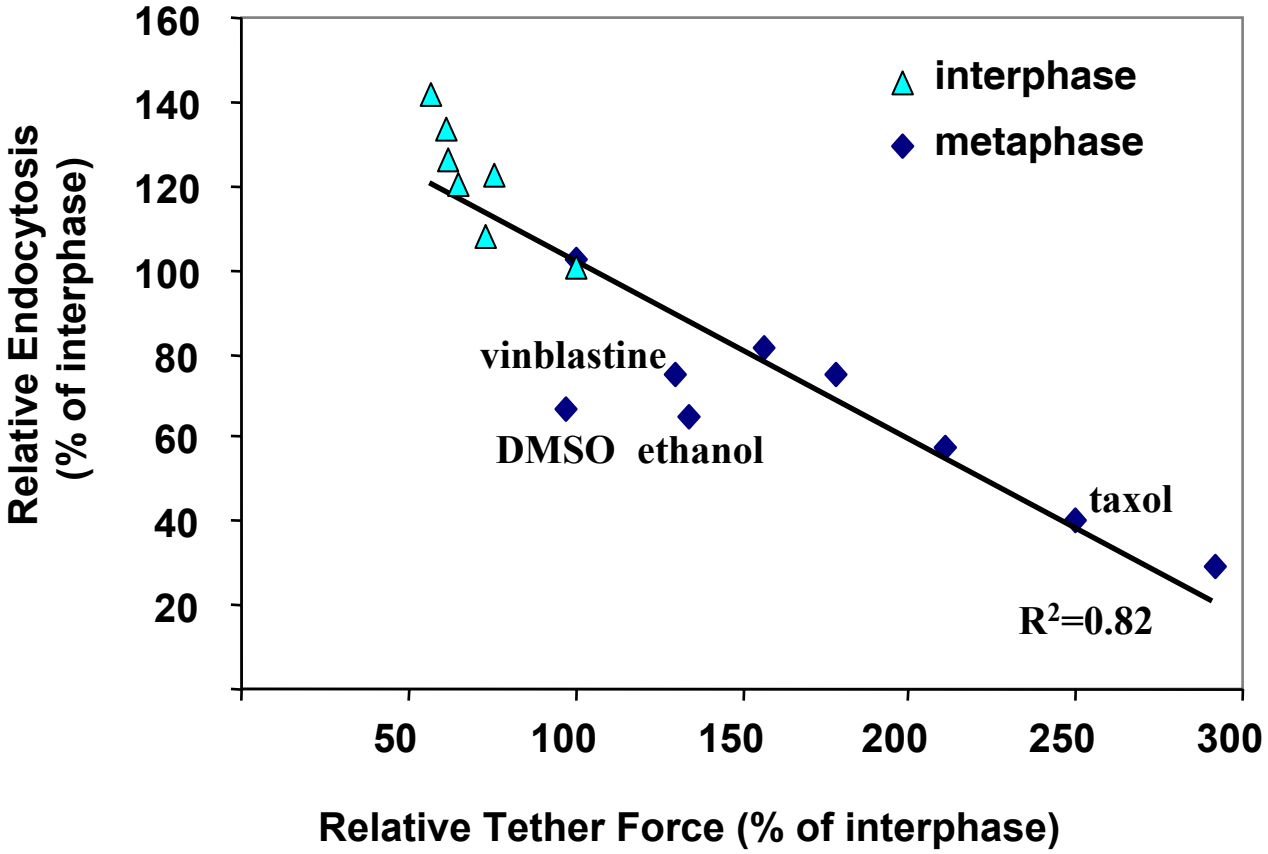




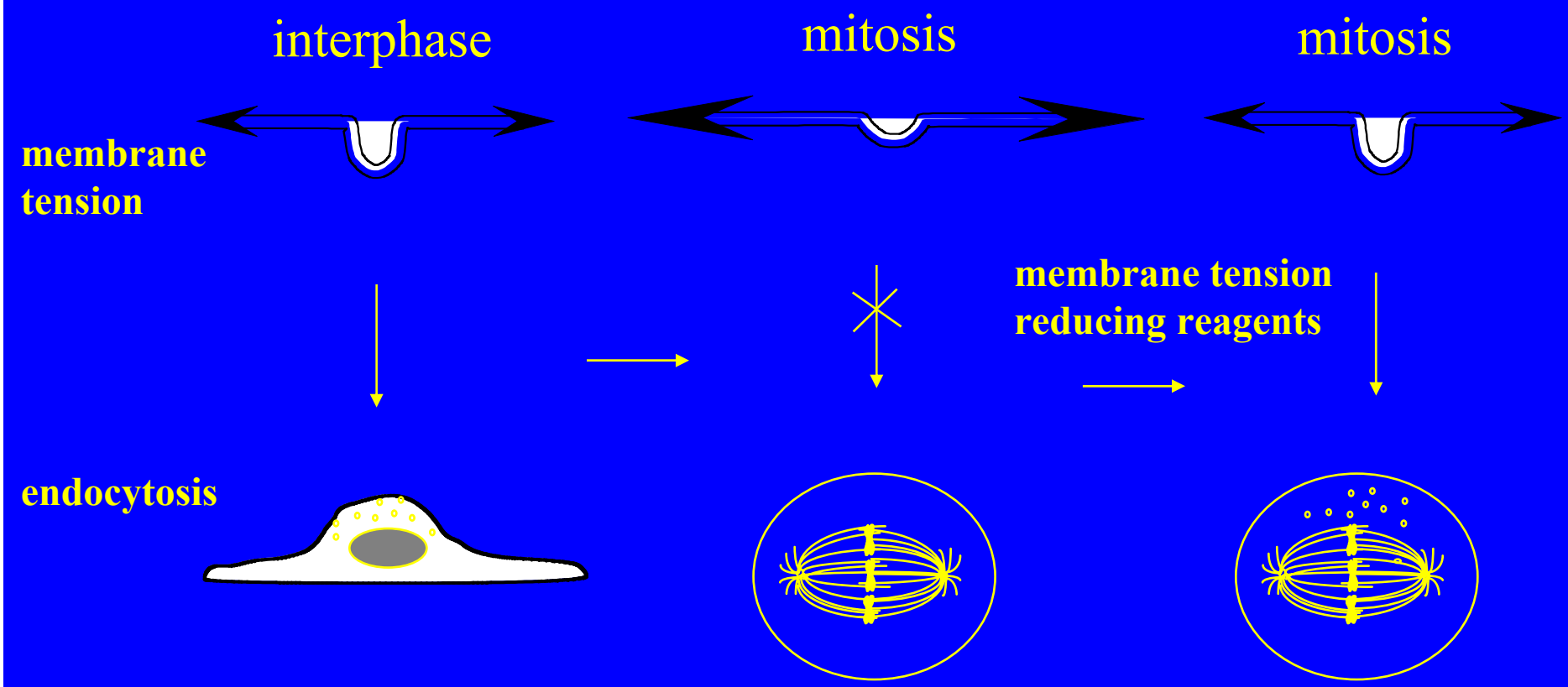
When membrane tension in metaphase cells is adjusted to the interphase level with detergent addition, the endocytosis rate reaches the interphase level.

Raucher & Sheetz (1999) *J. Cell Biol.* 144: 497

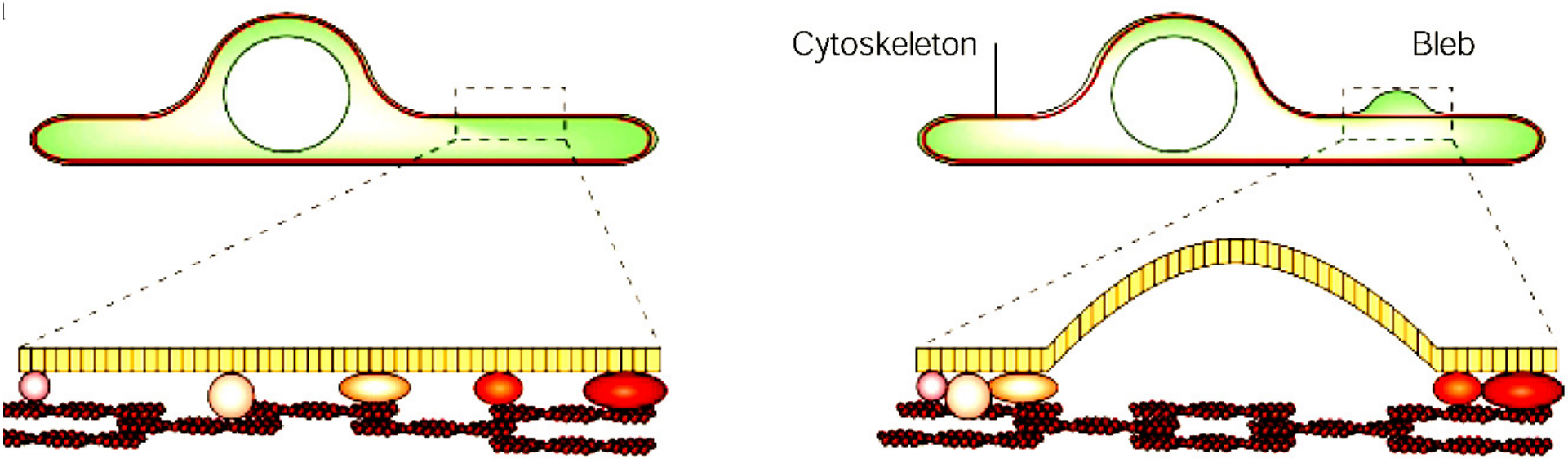
Correlation of Relative Endocytosis Rate and Membrane Tension



Model for Regulation of Endocytosis during Mitosis

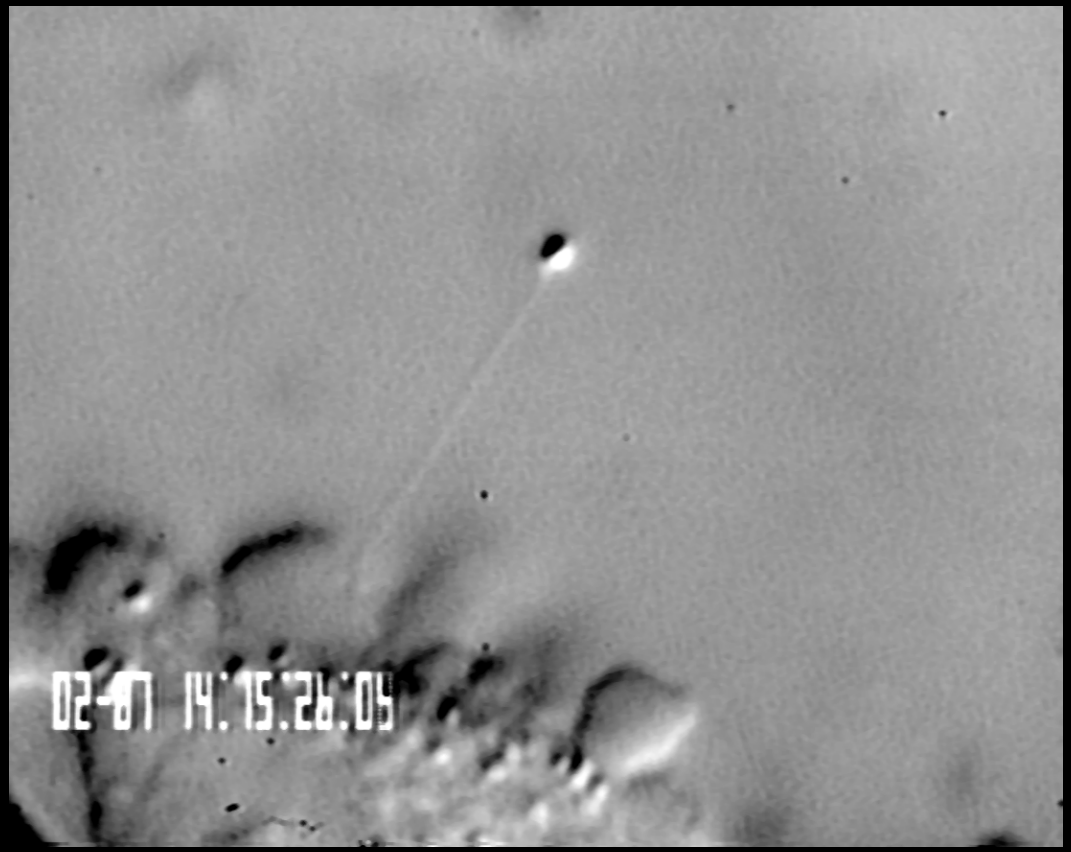


Bleb Formation Involves Separation of membrane from cytoskeleton

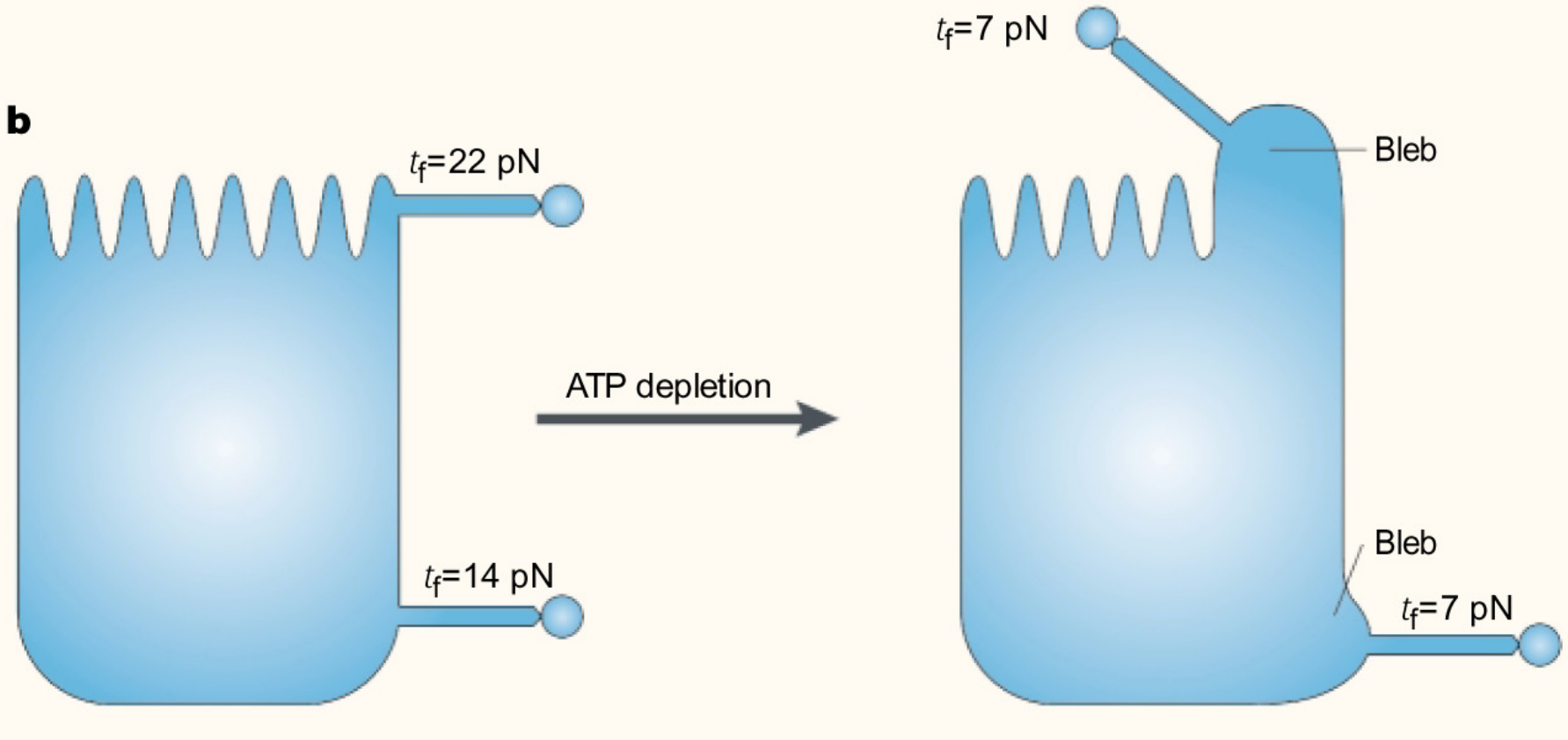


- Sheetz, MP. Nature Rev.CMB. 2:392-8(2001)

A tether from a bleb on a M2 cell



Tether Force over Cytoskeleton versus Blebs in Epithelial Cells



Dai & Sheetz (1999) Biophys. J. 77:3363

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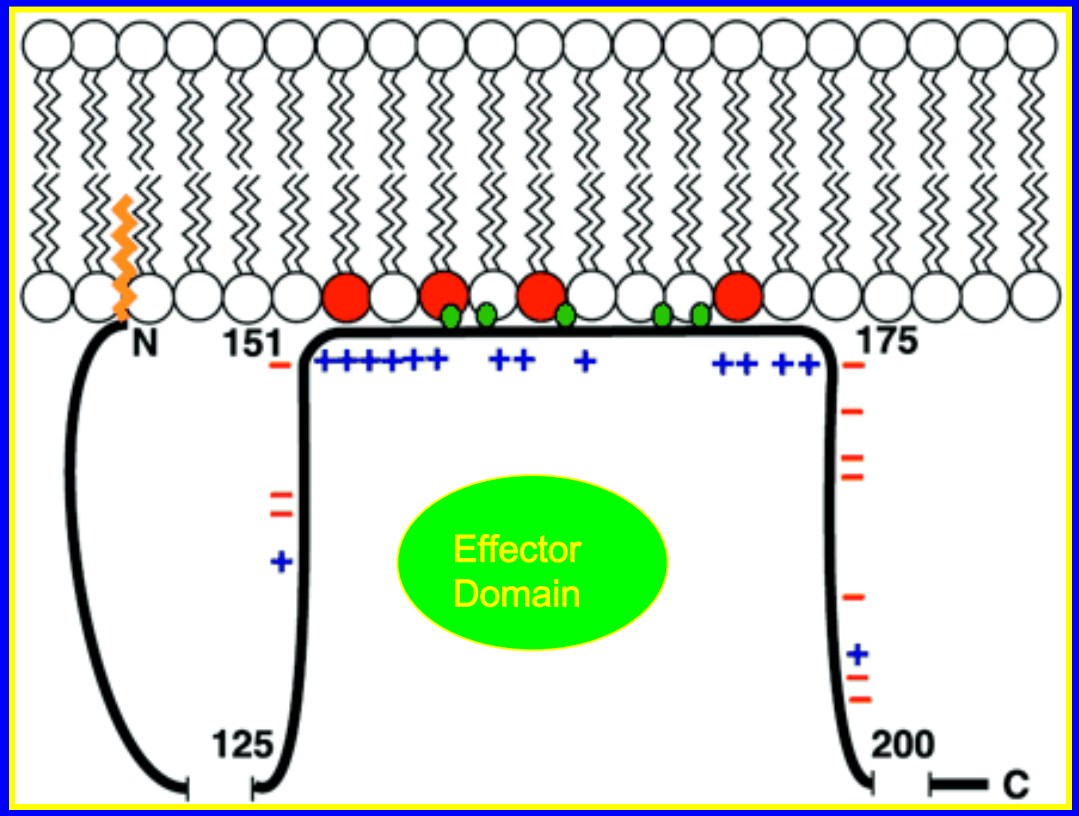
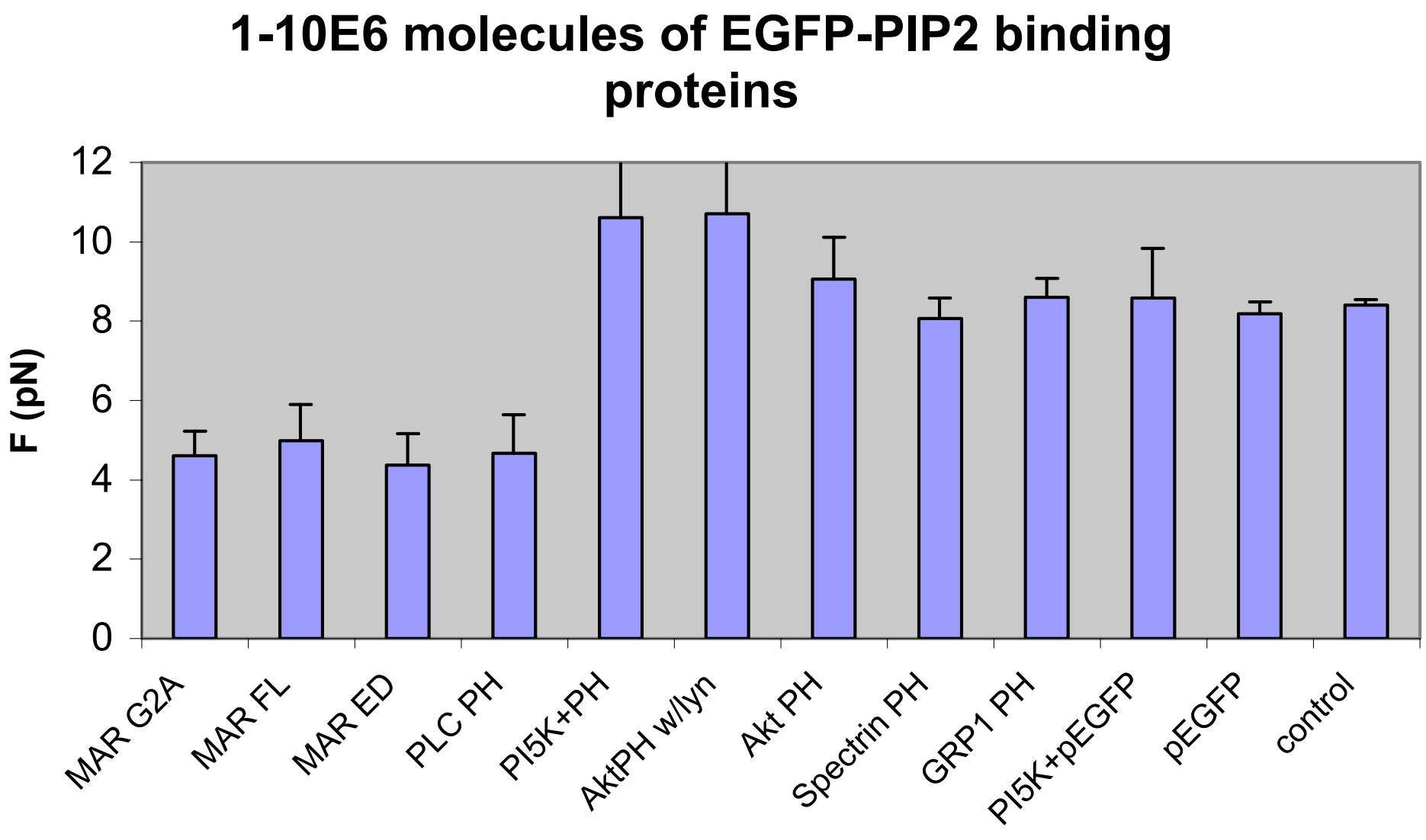
MARCKS (Myristilated Alanine Rich C Kinase Substrate)

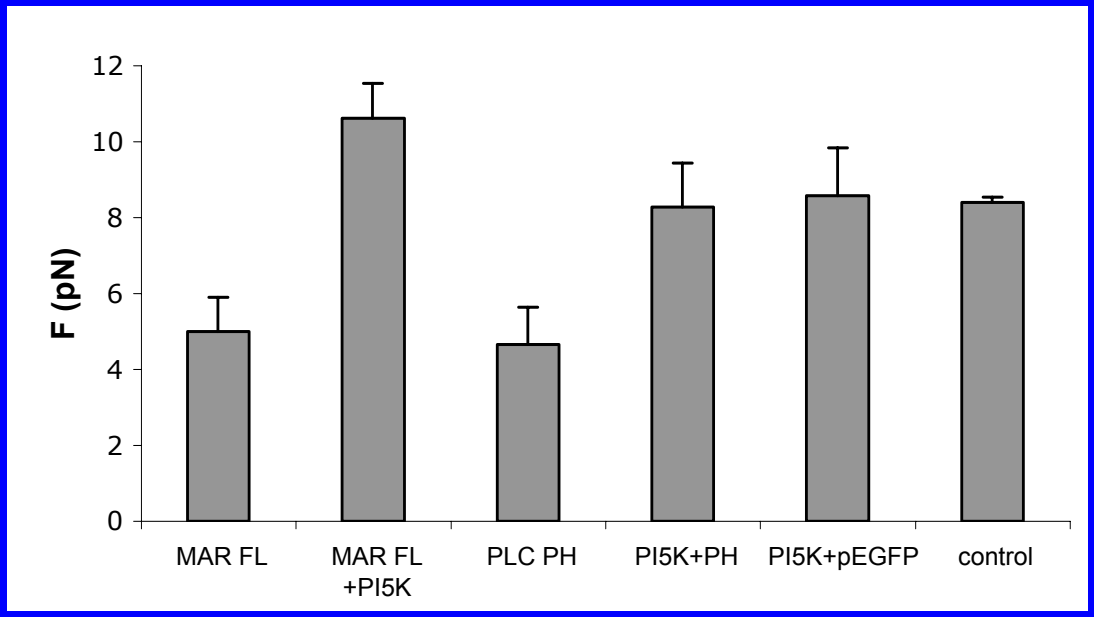
- Concentration present in cell at 10μM
- Binds 3 to 4 molecules PIP(4,5)2 with a K_D of 10^{-8}M
- Effector domain (AA 151-175) has +13 charge
- Competes w/ PLCδ PH for PIP(4,5)2
- Other functions: actin cross-linking protein and as a 'scaffolding' protein between the PM and the cytoskeleton

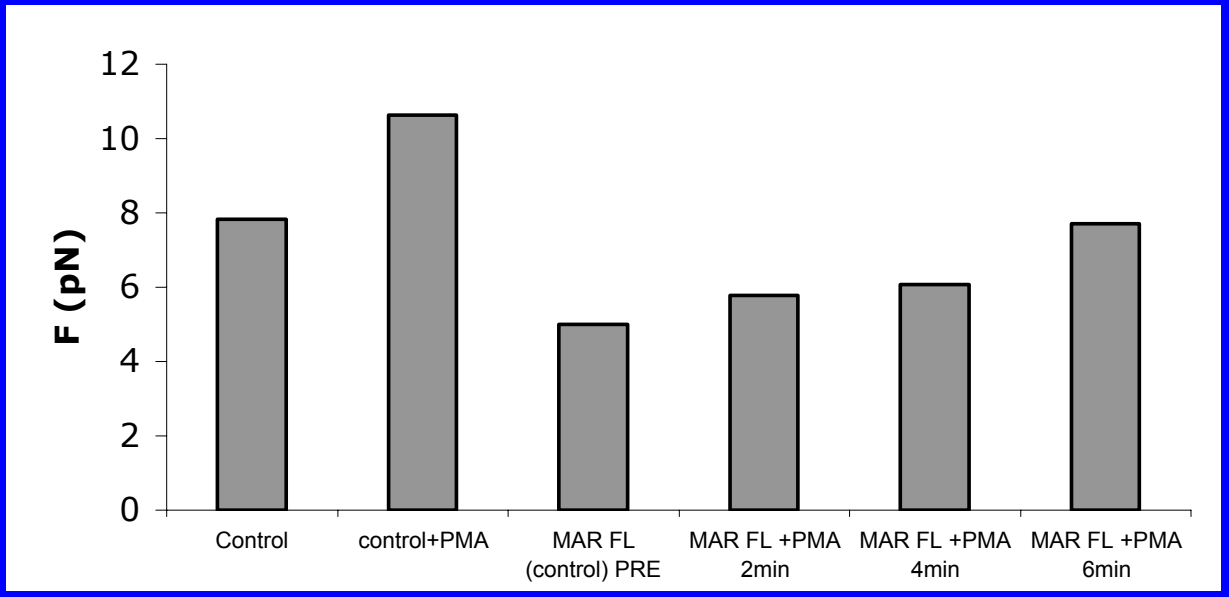


MARCKS, PLCδ-PH, Are Equally Effective in Reducing Tether Force



PI5 Kinase & PKC Restore Normal Adhesion



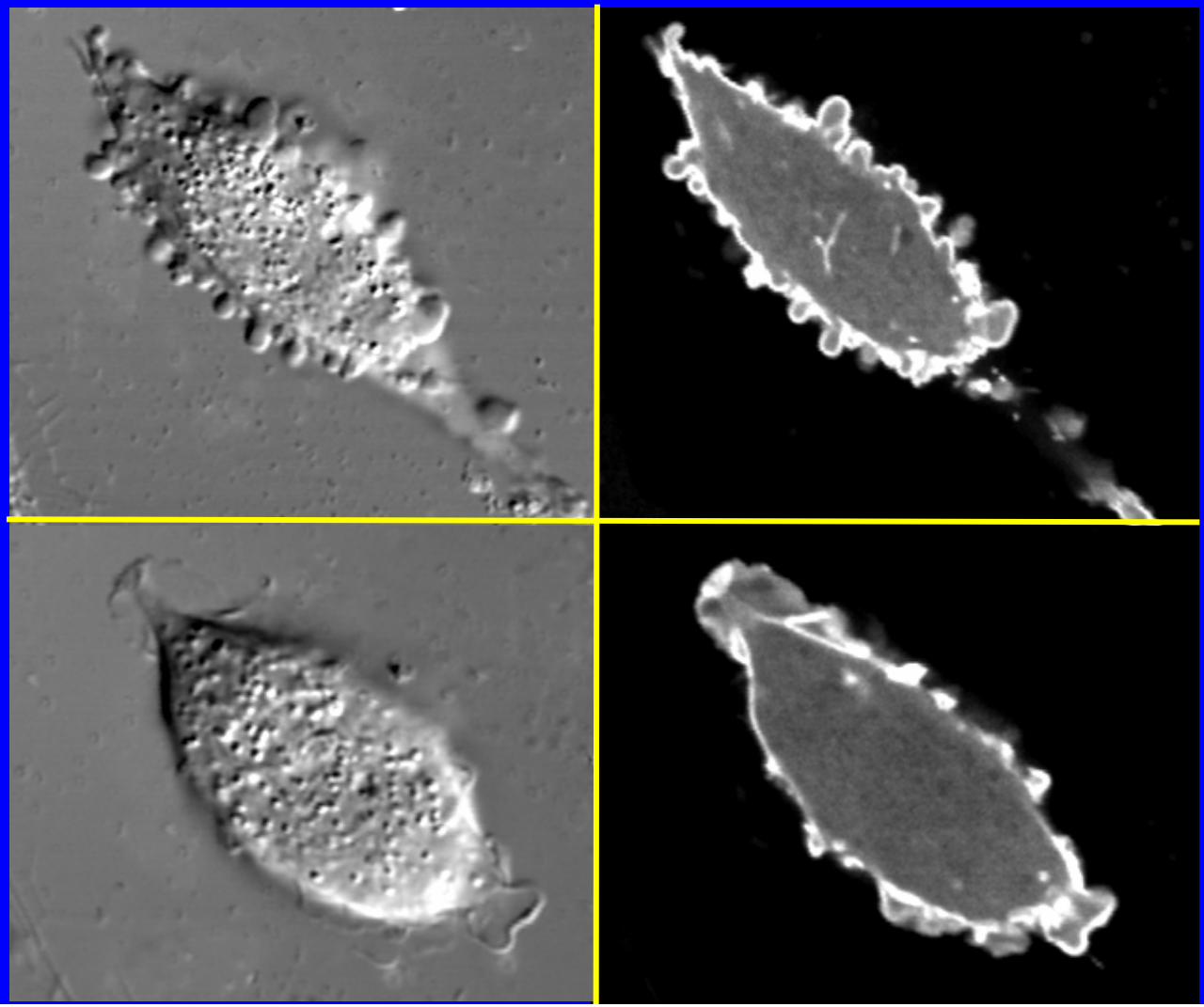


Protein Kinase C Activation Reverses PH Domain Blebs

Pre

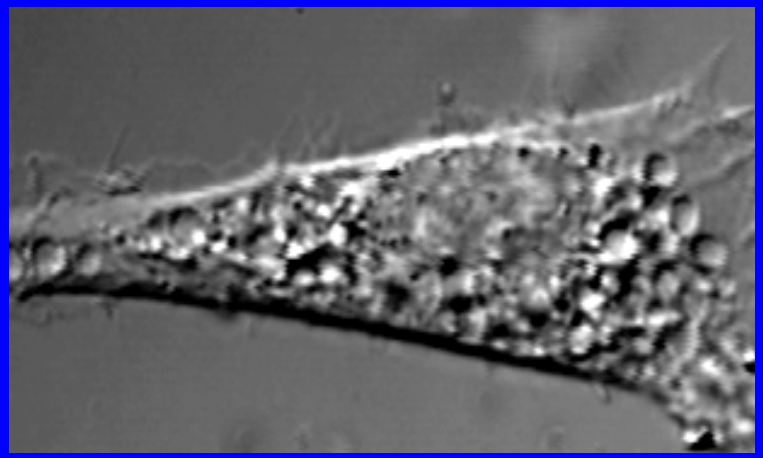
+ PMA

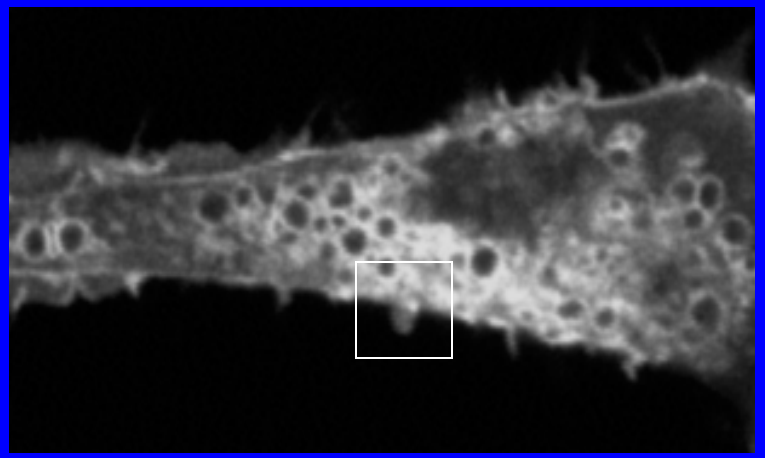
1 min



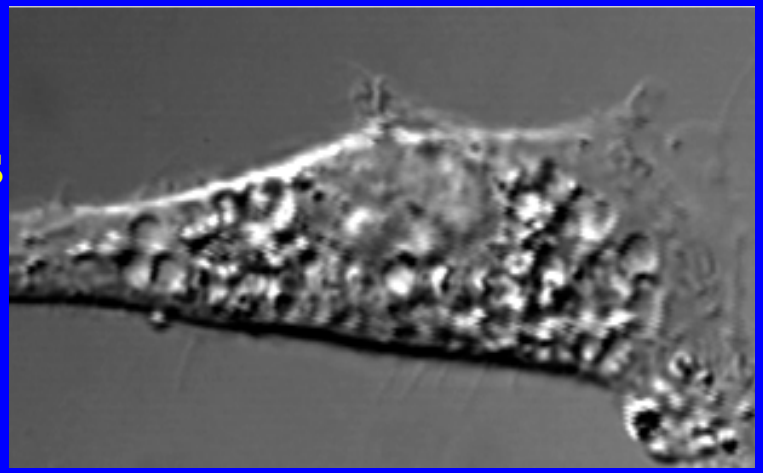
MARCKS Causes Small Blebs Reversed by PMA

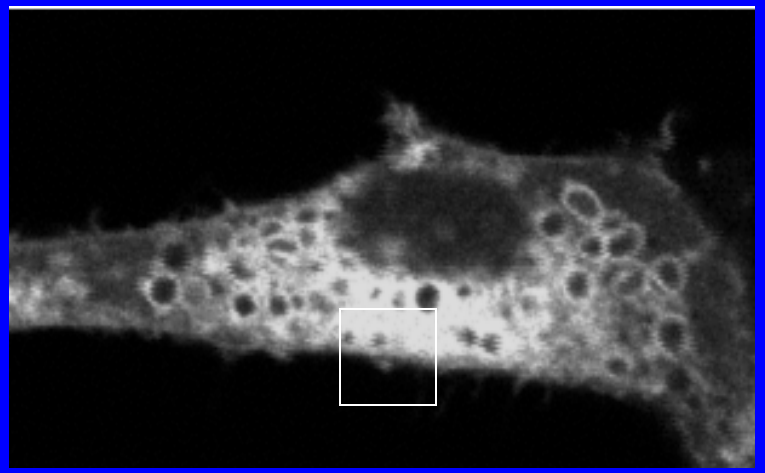
MARCKS



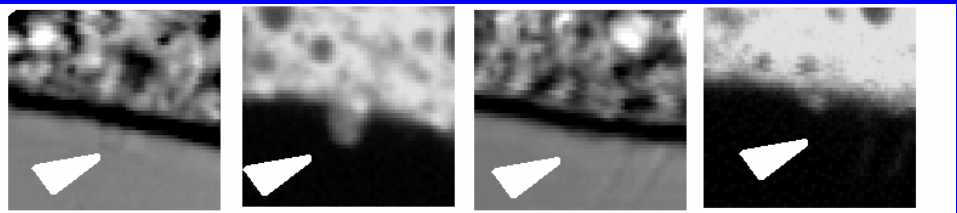


MARCKS + PMA

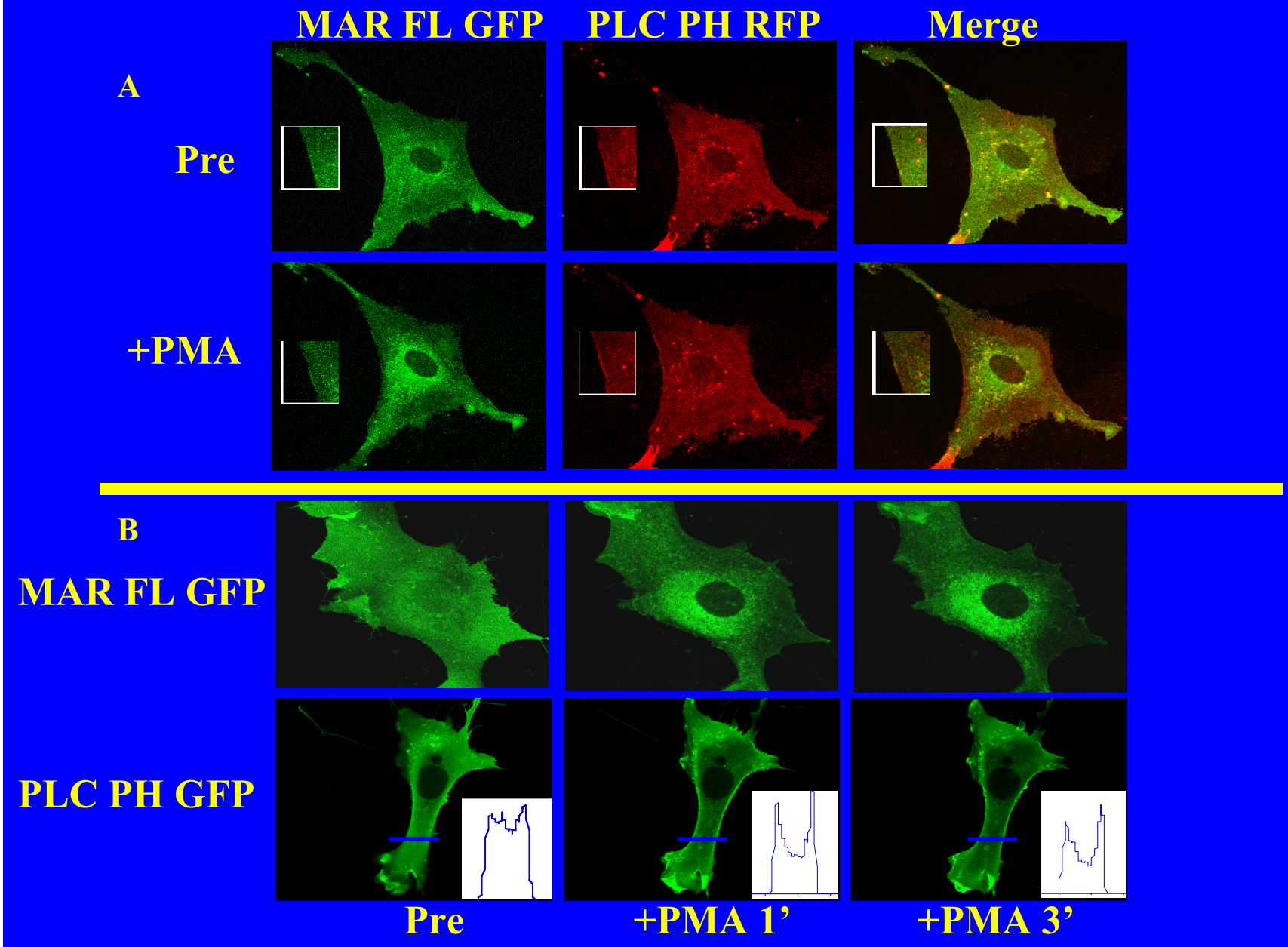




Enlarged Region

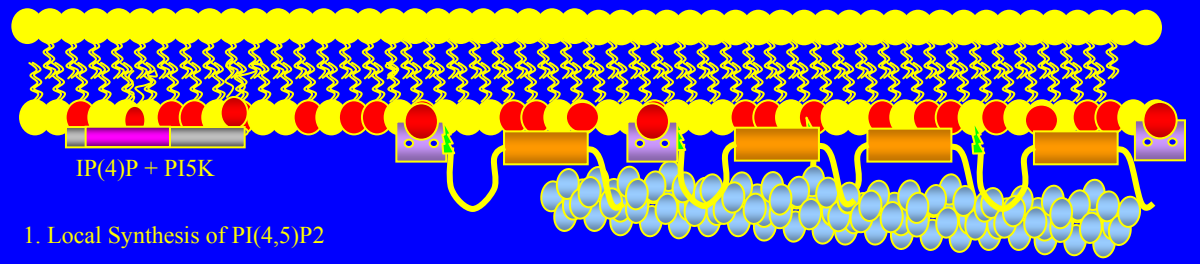


MARCKS & PH Domains Move Oppositely with PKC

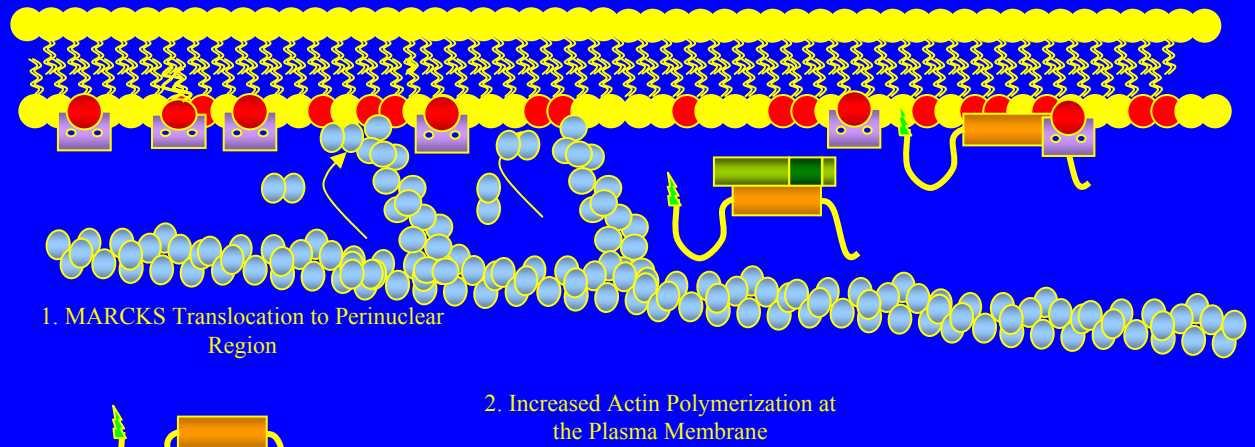


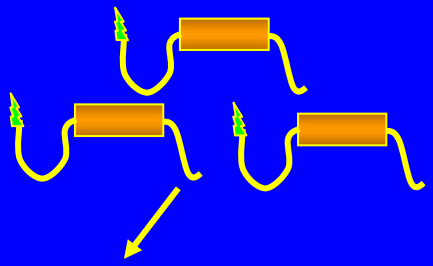
MARCKS Sequesters PIP2 Released by PKC

A

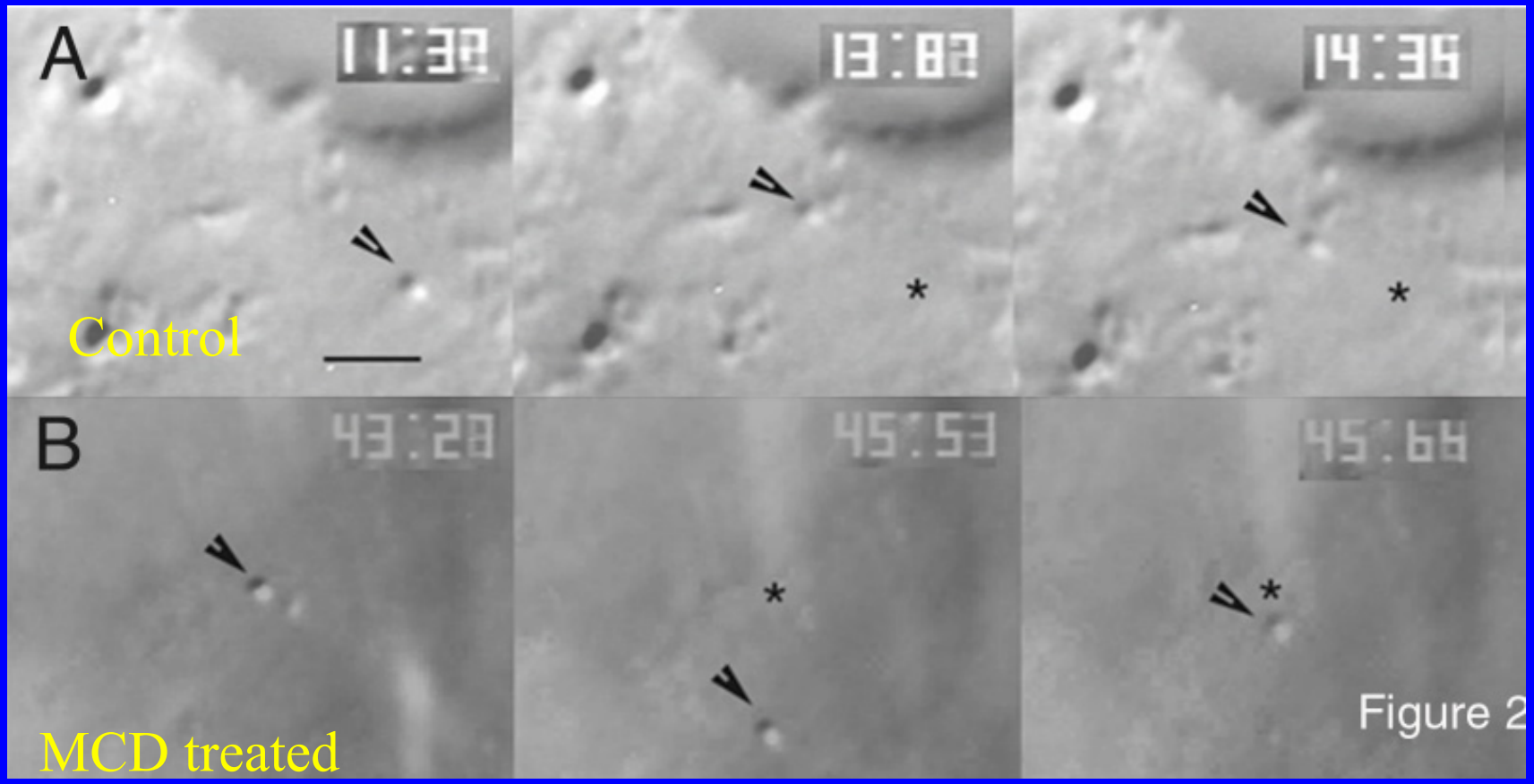


B





Cholesterol Depletion Increases Recoils and Decreases Diffusion



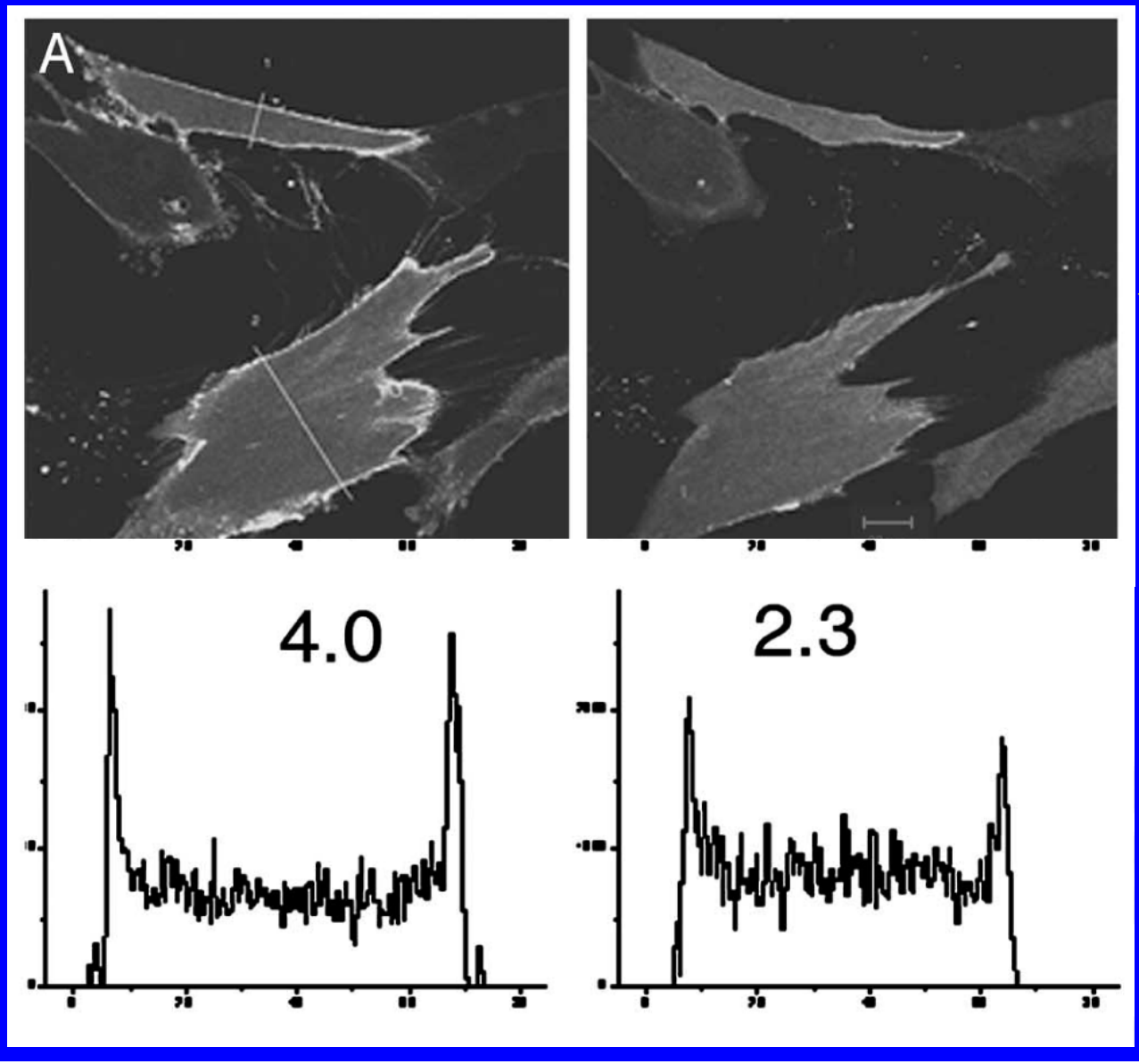
Kwik et al., PNAS 100:13964-9 (2003).

GFP-PLC-PH Leaves PM in 20’ of MCD

Before

After 20’

10mM MCD

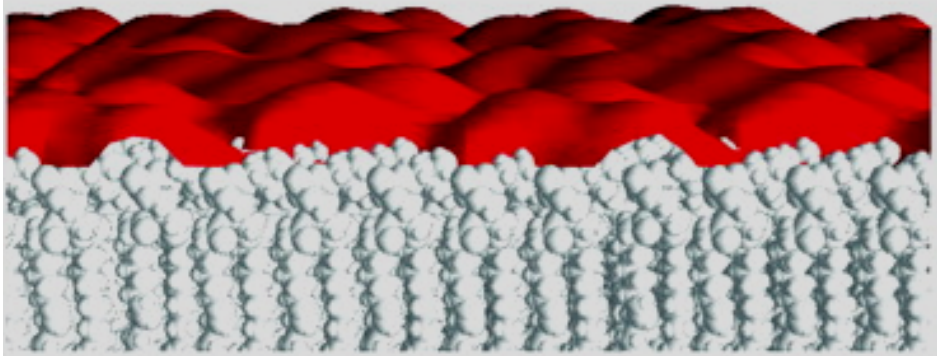


Intensity

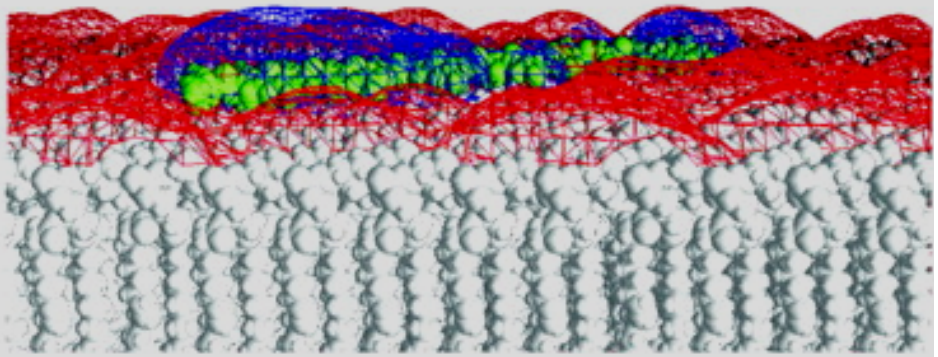
GFP-PH

Surface Potential of PS(20%) or PIP2 (1%) with MARCKS ED (0.1 M NaCl)

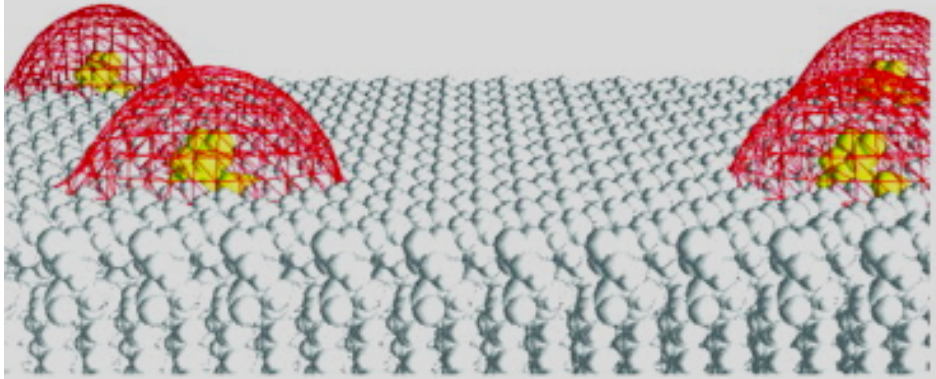
PS (20%)



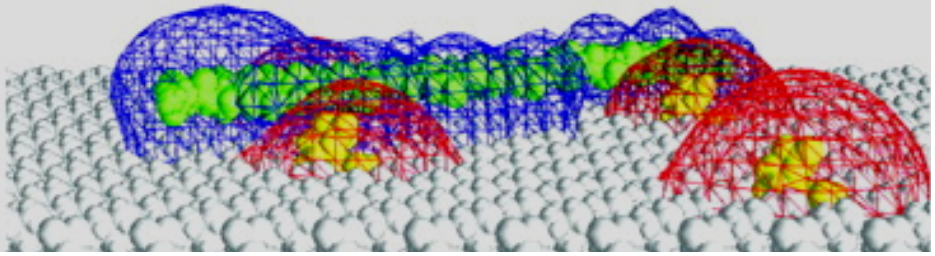
PS + MARCKS ED



PIP2 (1%)

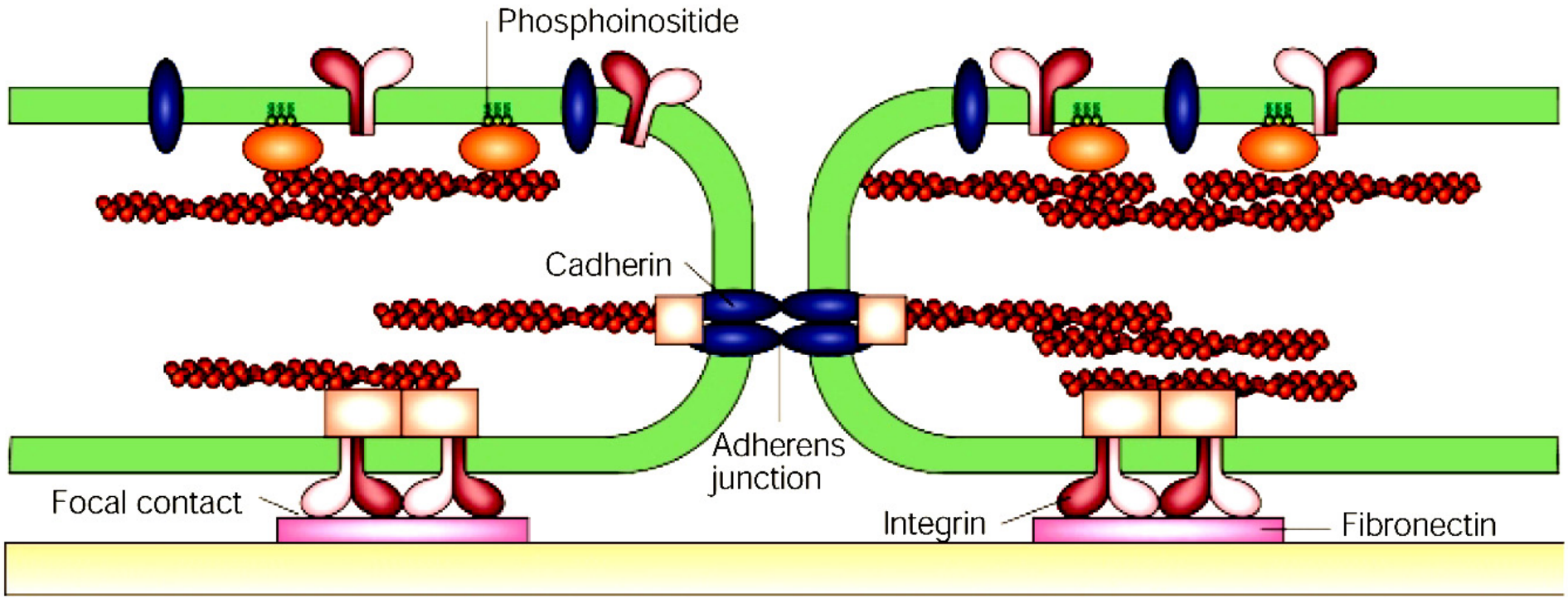


PIP2 + MARCKS ED



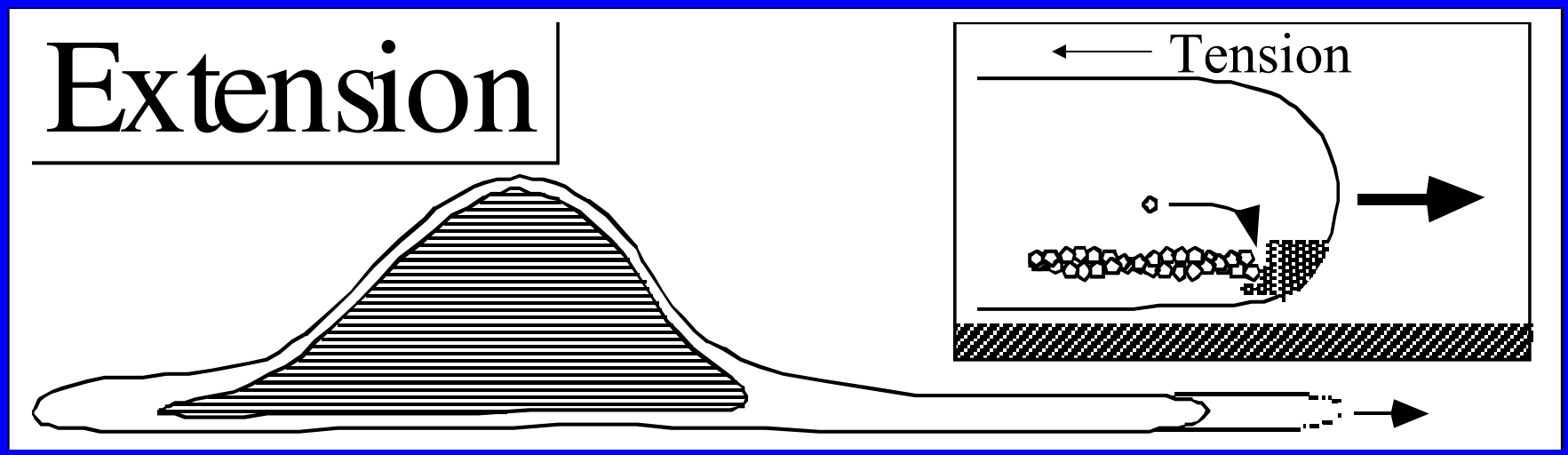
Wang et al., JBC 277:34401 (2002).

Model of Membrane Structure



From Sheetz, Nature Rev CMB 2:392-8(2001)

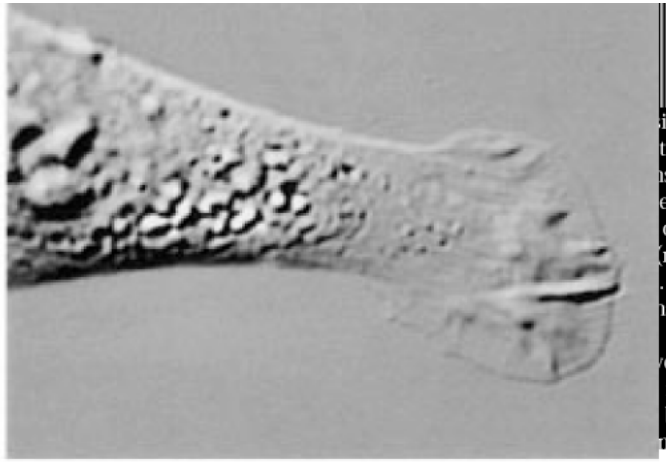
Apparent Membrane Tension Resists Extension by Actin and Tension α [PIP2]

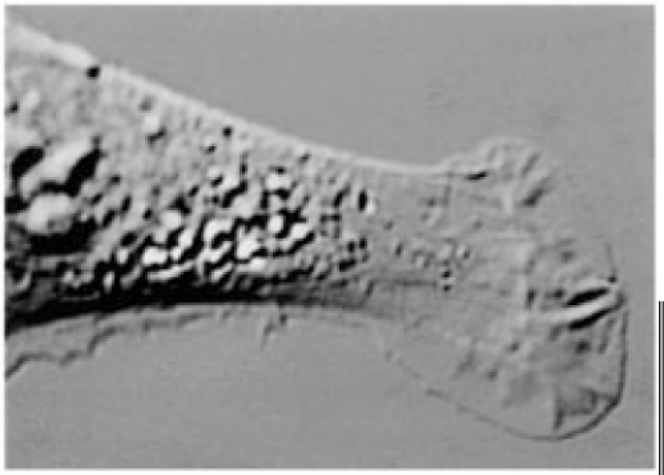


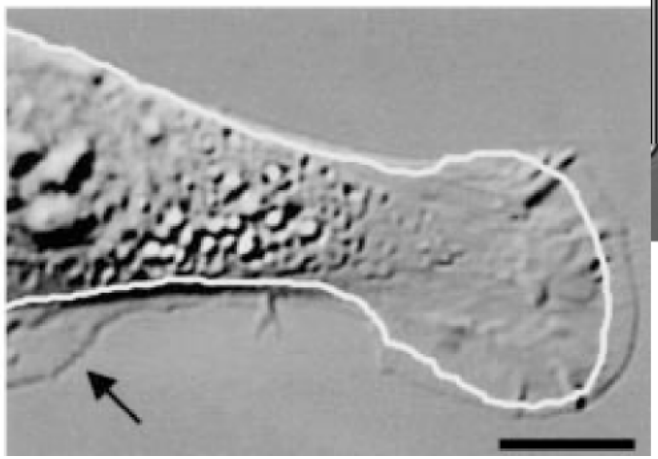
Reviewed: Sheetz, Nature Rev. MCB. 2:392 (2001)

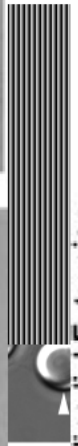
counted, normalit
number of lame

Reagents that Increase Membrane Area Increase Lamellipodial Extension

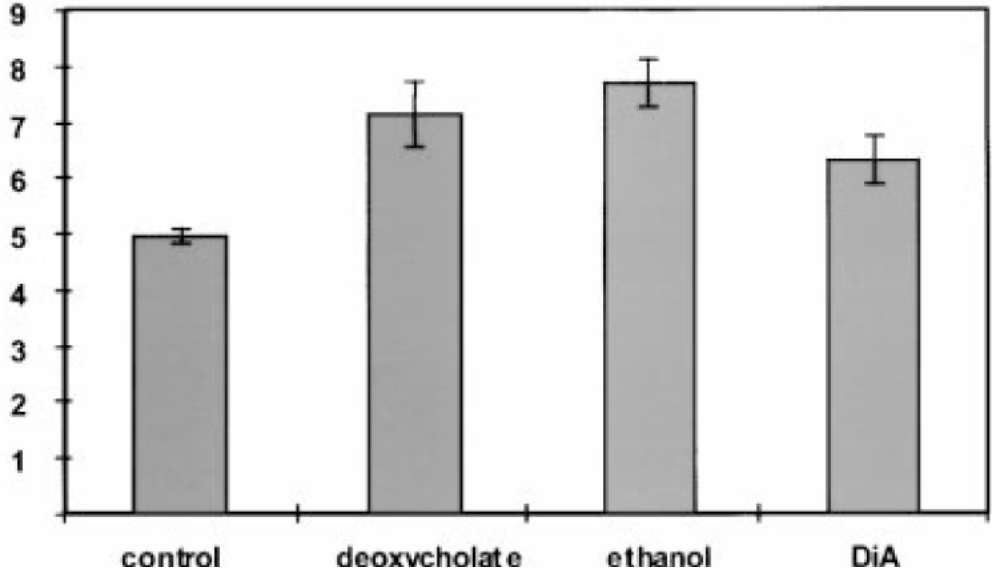


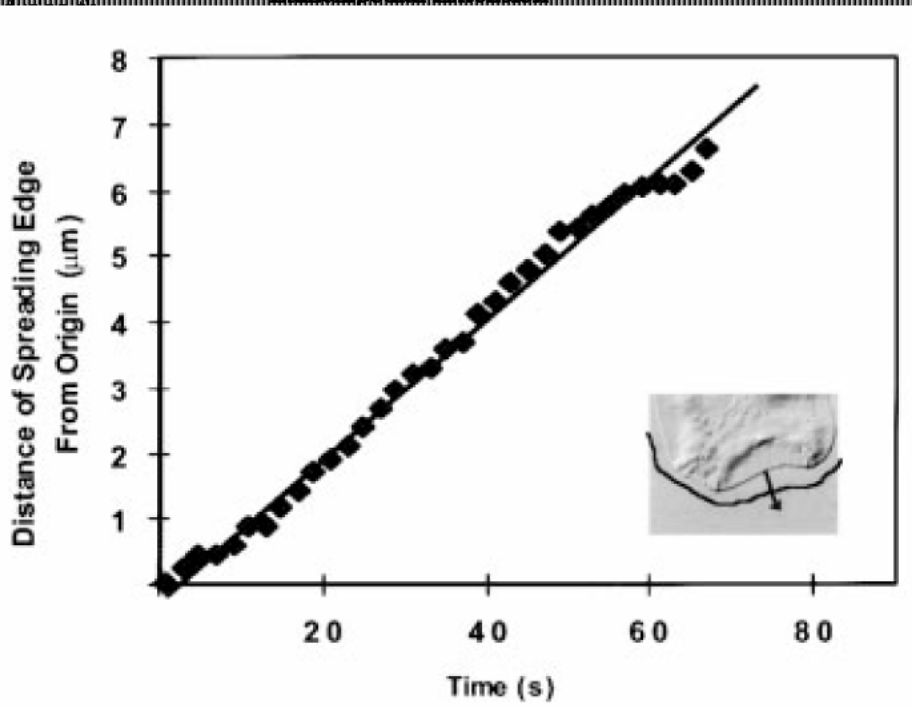


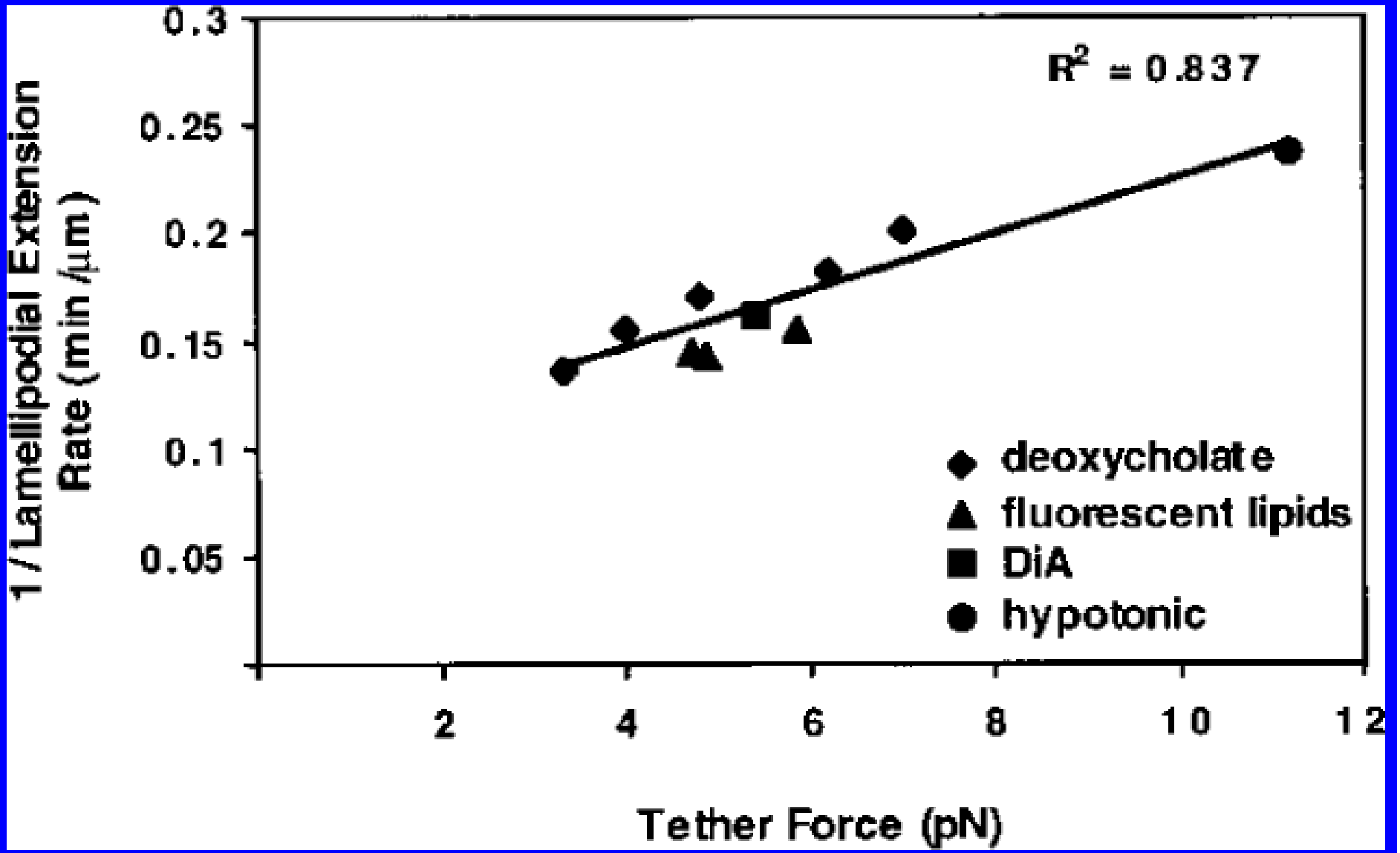




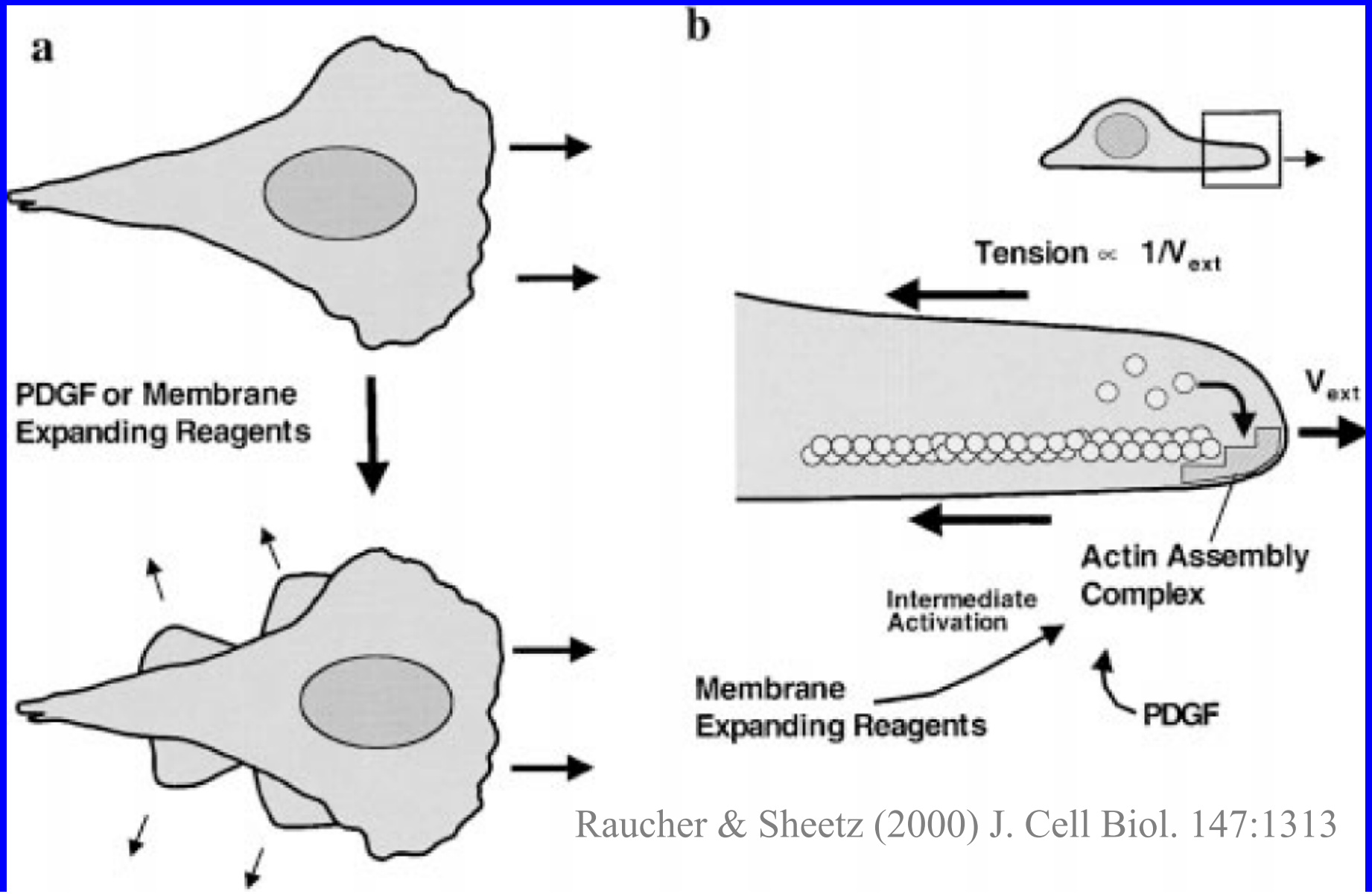
Lamellipodial Extension Rate(μm/min)






0.3
0.25
0.2
0.15
0.1
0.05
1 / Lamellipodial Extension Rate (min /μm)
R² = 0.837
deoxycholate
fluorescent lipids
DiA
hypotonic
2
4
6
8
10
12
Tether Force (pN)

Membrane Load Alters Activation and Rate of Actin Assembly



Raucher & Sheetz (2000) J. Cell Biol. 147:1313

Membrane Tension: Physical Control of Cell Functions

- Adhesion of membrane to the cytoskeleton develops tension that provides general regulation of endocytosis rate (surface-area/volume ratio), motility, and resealing
- Model of lipid-dependent membrane-cytoskeleton adhesion
- PIP2 is major lipid controlling membrane-cytoskeleton adhesion
- Tension is too low to activate stretch channels

Acknowledgements

Sheetz Lab

Drazen Raucher
Jianwu Dai
Catherine Galbraith
Goetz Wichert
Dan Felsenfeld
Julia Sable
Daniel Choquet
Guoying Jiang
Ben Dubin-Thaler
Adam Meshel
Yasuharo Sawada
Masako Tamada

Duke University
Dr. Robert Hochmuth

Stuart McLaughlin
(SUNY Stonybrook)

Michael Edidin

North Carolina State University
Dr. Paul Franzon
David Nackashi